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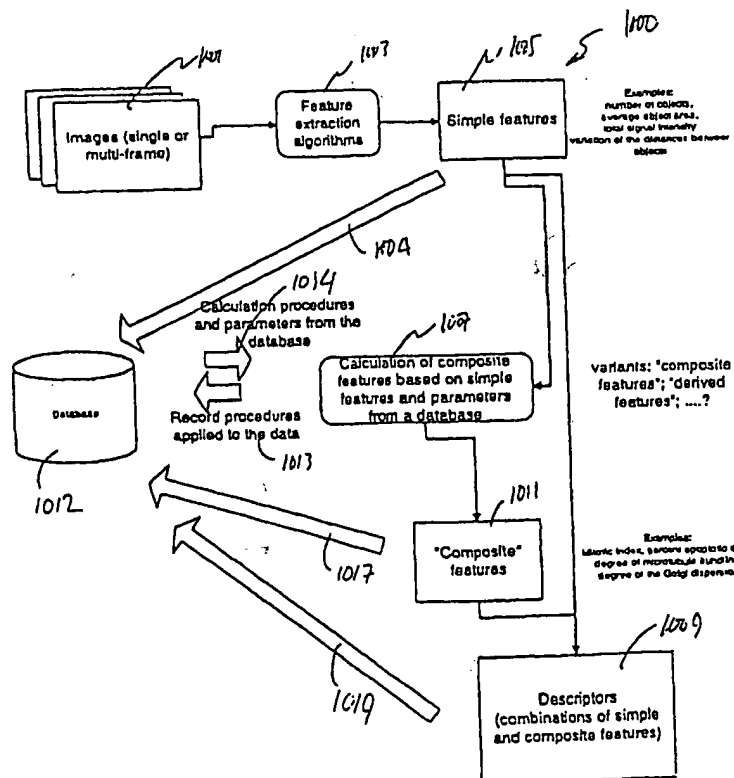
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(57) Abstract

Techniques for using information technology in therapeutics or drug discovery. In an exemplary embodiment, techniques for determining information about the properties of substances based upon information about structure of living or non-living cells exposed to substances are provided. A method according to the present invention enables researchers and/or scientists to identify promising candidates in the search for new and better medicines or treatments using, for example, a cellular informatics database. The present invention further teaches a system for acquiring knowledge from cellular information. The system has a database 1012 comprising a database management module ("DBMS"). The system also has a variety of modules, including a population module coupled to the DBMS for categorizing and storing a plurality of features (e.g., cell size, distance between cells, cell population, cell type) from an image acquisition device into the database. The system has a translation module coupled to the DBMS for defining a descriptor from a set of selected features from the plurality of features. In a specific embodiment, the descriptor is for a known or unknown compound, e.g., drug. A prediction module is coupled to the DBMS for selecting one of a plurality of descriptors from known and unknown compounds from the database based upon a selected descriptor from a selected compound. The selected compound may be one that is useful for treatment of human beings or the like.



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PATENT APPLICATION
METHOD AND APPARATUS FOR
PREDICTIVE CELLULAR BIOINFORMATICS
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10 computer codes, which may be used to implement aspects of the present invention. Assignee of the present invention reserves all rights with respect to these codes and provides notice herein. Notice is hereby given © Cytokinetics, Inc. 1999.

BACKGROUND OF THE INVENTION

15 The present invention provides techniques for information management using a database platform. More particularly, the present invention provides a system including computer code that couples to a database device. The system provides for image capturing of living, dead, or fixed cells or cell fractions used to identify information about substances used on the cells or information about the cells themselves. Accordingly, the present invention can enable researchers and
20 scientists to identify promising candidates in the search for new and better medicines, for example, in drug discovery and development. The principles enumerated herein may, with equal facility, be applied to other applications, including but not limited to use in environmental applications such as determining chemical toxicities and other non-pharmaceutical toxicology uses.

25 For a long time, researchers in the pharmaceutical field have sought for better ways of searching for substances possessing properties that make them suitable as medicines. In the early days, researchers generally relied upon extracts from plants, dyes, and microbiological extracts for such substances. Examples of such substances include the pain reliever aspirin, the anti-cancer drug paclitaxel (brand
30 name TaxolTM), and the heart medication called digoxin. The number of useful medicines has generally been limited.

Purified substances having desirable bio-active properties are also often difficult to discover. Advances in traditional organic chemistry and more recently the rapid chemical synthesis methods often referred to as combinatorial chemistry have increased the number of compounds that researchers test for biological activity. Originally, substances were often initially tested on animals or humans to determine their biological activity. While results from such tests may identify a good drug candidate, they are often time consuming and costly, thus a limited number of substances can be tested. Therefore, pharmaceutical companies have turned to testing their ever-increasing libraries of substances against isolated proteins (drug targets) in biochemical assays that can be carried out at high throughput and low cost. It should be noted that the substances need to be tested in numerous protein tests, each customized for a particular drug target. Therefore, although each protein test may be run at a high-throughput, the design of multiple protein tests can be time-consuming. Substances deemed promising based on results from the protein tests are then tested in lower throughput cellular and animal tests.

There have been some attempts to use image acquisition techniques to screen a large number of substances based upon biological cell information. One such attempt is described in International Application No. WO 98/38490 in the names of Dunlay, et al. Dunlay et al. generally describes a conventional image acquisition system. This conventional system collects and saves images based on certain criteria that are predefined, not on a fixed area of an imaging surface. Additionally, the conventional system has poor lighting design, which makes image processing for multiple cells difficult. Furthermore, the conventional system is not designed for capturing, populating and utilizing a large database design. The conventional system is designed for customized cellular assays, not as a tool for generation of a cellular informatics database. Without such database capabilities the conventional system cannot be used for screening, analyzing, and comparing large quantities of cells from multiple experiments on multiple days in a predictive, efficient and cost effective manner.

What is needed is a rapid assay to assess the activity of compounds against multiple drug targets simultaneously in a cellular context. What is also needed are techniques for finding the effects of substances on cell function based upon searching and analyzing cellular information.

SUMMARY OF THE INVENTION

According to at least one embodiment of the present invention, techniques for determining information about effects of potential substances on cells are provided. In another exemplary embodiment, the present invention provides a novel system including hardware, computer codes, user interfaces, and a database for acquiring, storing and retrieving cellular and substance information. The cells can include living, dead, or fixed cells or fractions of cells. The present invention enables, *inter alia*, researchers and/or scientists to identify promising candidates in the search for new and better medicines or treatments using, for example, a cellular informatics database.

According to the present invention, a computer program for identification and verification of biological properties of substances can include code that causes a sample of a substance to be administered to a cell. The code determines one or more features for two or more cell components, or markers, in the presence of the substance. The code can form one or more descriptors from the features. Descriptors can be formed by combining features of two or more cell components as identified using the markers. The code can then search one or more descriptors obtained from prior administered substances upon cells in order to locate descriptors having a relationship to the descriptors noted for the substance under study. The code predicts properties of the administered substance based upon the properties of the prior administered substances using the relationship between the descriptors. The code can provide for identifying properties of substances based upon effects on cell characteristics. Candidate drug mechanisms of action, potency, specificity, pharmacodynamic, and pharmacokinetic parameters, toxicity, and the like can be used as substance properties.

In a specific embodiment, the present invention provides a system for acquiring knowledge from cellular information. The system has a database comprising a database management module ("DBMS"). The system also has a variety of other modules, including a population module that is coupled to the DBMS and serves to categorize and store a plurality of features (including but not limited to cell size, distance between cells, cell population, as well as sub-cellular features such as organelle location, protein location and sub-cellular constituent location and

movement) from an image acquisition device into the database. The system has a translation module coupled to the DBMS for defining a descriptor from a set of selected features from the plurality of features. In a specific embodiment, the descriptor is for a known or unknown compound, e.g., drug. A prediction module is coupled to the DBMS for selecting one of a plurality of a descriptors from known and unknown compounds from the database based upon a selected descriptor from a selected compound. The selected compound may be one that is useful for treatment of human beings or the like.

In a specific embodiment, the present invention provides a system for populating a database with cellular information. The system includes a cell holder (e.g., multi-well plate, chip, microfluidic assembly, or other cell chamber) comprising a plurality of sites in a spatial orientation. Each of the sites is capable of holding a plurality of cells to be imaged. Note – the light guide is one embodiment, but we don't want to be limited to it.

According to one embodiment, the present system also has an illumination apparatus including a liquid light guide operably coupled to the imaging device for highlighting the plurality of cells in a relatively even spatial manner for image capturing and measurement purposes. Still further, the liquid light guide allows sub-elements (e.g., filter, lamp) of the illumination apparatus to be placed at a remote location to prevent mechanical interference of the cell holder during image capturing. Alternative lighting methodologies may, with equal facility, be implemented.

The system also has an image-capturing device (e.g., charge coupled device camera, translation stage, shutter, microscope, software, shutter control) coupled to a computing device (e.g., computer, network computer, work station, analog computing device, on-board image-processor, and laptop). The image-capturing device is adapted to capture at least one image in at least one of the plurality of sites. One some embodiments, multiple images can be captured, where each image represents a different cell component (or portion). The image-capturing device can be adapted to convert the image into a digital representation, which highlights the feature or features of the one site.

A database storage device (e.g., relational database, object oriented database, mixed object oriented database) includes a database management element. The

database is coupled to the image capturing device. In a specific embodiment, the present system includes modules for feature extraction, generation of descriptions, and data preparation and analysis.

In a specific embodiment, the present invention provides a novel
5 system for determining an effect of a manipulation of a cell using one or more image frames. The system has a plate comprising a plurality of sites in a spatial orientation. Each of the sites is capable of holding a plurality of cells to be imaged. The system also has an image capturing device to capture a plurality of images of at least one site from the plurality of sites. The image capturing device is coupled to the computing
10 device. The system also has an image processing device to combine the plurality of images of at least one site or plurality of sites. The image processing device is operably coupled to the plate. An image processing device is also included. The image processing device can be adapted to form a digitized representation of the plurality of images from the site or plurality of sites. Furthermore, the system has a
15 database storage device comprising a database management element. The database can be adapted to retrieve the descriptor or descriptors of the plurality of features from the computing processing device and storing them in a selected manner.

In a specific embodiment, the present invention provides a system for capturing cellular information. The system also has an image acquisition system
20 comprising a charged coupled device camera adapted to capture an image of a plurality of manipulated cells in various stages of the cell cycle. The stages of the cell cycle are currently understood to include interphase, G0 phase, G1 phase, S phase, G2 phase, M phase, prophase, prometaphase, metaphase, anaphase, and telophase. The principles of the present invention specifically contemplate the application thereof on
25 additional cell cycle stages when and if they are identified.

An optical source is coupled to the image acquisition system for highlighting the plurality of manipulated cells in the various stages of the cell cycle. The illumination apparatus provides for an acquisition of the image of the plurality of manipulated cells. In a specific embodiment, the illumination apparatus has a liquid
30 light guide coupled to a light source at a remote location.

A variety of user interfaces are utile for accessing the several features of the present invention. Those having ordinary skill in the art will appreciate that different user interfaces may be required to support different research scenarios. The

present invention specifically contemplates the utilization of a wide variety of user interfaces.

Numerous benefits are achieved by way of the present invention over conventional techniques. The present invention can provide techniques for predictive cellular bioinformatics that can streamline a number of important decisions made in the drug discovery industry. The present invention can be implemented using off the shelf hardware including databases. In other aspects, the present invention can find useful information about substances as well as cells or portions of cells. Furthermore, the present invention can acquire more than one feature using more than one manipulation. Moreover, the present invention can provide information about a wide variety of cellular information that is not conventionally available. This information includes information about different cell components, e.g., nuclei and Golgi apparatus. Still further, the present invention provides an automated or semi-automated technique for acquiring images and populating a database. The present database can be combined with others such as genomics, and the like. Moreover, the present invention can be implemented to predict, *inter alia*, a mechanism of action, toxicity, target validation, and pre-clinical disease model.

A further understanding of the nature and advantages of the invention herein may be realized by reference to the remaining sections of the specification and the attached drawings.

BRIEF DESCRIPTION OF THE DRAWING

For more complete understanding of the present invention, reference is
5 made to the accompanying Drawing in the following Detailed Description of the
Invention. In the drawing:

Fig. 1 is a simplified system diagram according to an embodiment
according to the present invention;

Figs. 1A-1B are more detailed diagrams of database systems according
10 to embodiments of the present invention;

Fig. 2 is a simplified block diagram according to an alternative
embodiment according to the present invention;

Figs. 3-6 are simplified diagrams of system elements according to
embodiments of the present invention;

15 Figs. 7A-7K illustrate representative block diagrams of simplified
process steps in a particular embodiment according to the present invention;

Fig. 8A-8F illustrate representative quantified descriptors of effects of
manipulations on images of cells in a particular experiment;

Fig. 9 illustrates example images for different types of morphologies in
20 a particular experiment;

Fig. 10 illustrates a distribution of various morphologies in a cell
population responsive to drug concentration in a particular experiment;

Fig. 11 illustrates a graph of quantified features of effects of
manipulations on cells in a particular experiment;

25 Fig. 12 illustrates effects of external agents on cells in a particular
experiment;

Fig. 13 illustrates 4 panels for each marker for a plurality of A549 cells
in a particular experiment;

Fig. 14 illustrates 4 panels for each marker for a plurality of OVCAR-3
30 cells in a particular experiment;

Fig. 15 illustrates 4 panels for each marker for a plurality of OVCAR-3
cells at 20x in a particular experiment;

Fig. 16 illustrates 4 panels for each marker for a plurality of OVCAR-3 cells at 40x in a particular experiment;

Fig. 17 illustrates a representative input for a morphometric analysis program in a particular embodiment according to the present invention; and

5 Figs. 18-19 illustrate examples of the generation of pseudo-sequences and clustering in a particular embodiment according to the present invention.

Fig. 20 is a block diagram for a first research scenario;

Fig. 21 is a block diagram for a second research scenario; and

Fig. 22 is a block diagram for a third research scenario.

10 Reference numbers refer to the same or equivalent parts of the invention throughout the several figures of the Drawing.

DETAILED DESCRIPTION OF THE INVENTION

According to the present invention, techniques for determining information about manipulated cells or substances based upon living, fixed, or dead cell structures or portions of cells are provided. In an exemplary embodiment, the present invention provides a novel system including computer codes coupled to a database and user interfaces for acquiring, storing and retrieving such information. Other embodiments provide a novel image capturing system for providing digitized representations of live and dead cell structures or the like.

Fig. 1 is a simplified system diagram 10 of a cellular knowledge-based system according to an embodiment of the present invention. This diagram is merely an example and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. The present system 10 includes a variety of elements such as a computing device 13, which is coupled to an image processor 15 and is coupled to a database 21. The image processor receives information from an image capturing device 17, which image processor and image capturing device are collectively referred to as the imaging system herein. The image capturing device obtains information from a plate 19, which includes a plurality of sites for cells. These cells can be biological cells that are living, fixed, dead, cell fractions, cells in a tissue, and the like. The computing device retrieves the information, which has been digitized, from the image processing device and stores such information into the database. A user interface device 11, which can be a personal computer, a work station, a network computer, a personal digital assistant, or the like, is coupled to the computing device.

Fig. 1A is a simplified diagram of a database system 1000 according to an embodiment of the present invention. This diagram is merely an example and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize many other variations, modifications, and alternatives. Database system 1000 includes a variety of techniques for processing images from biological cells, e.g., fixed, living, and dead cells, and cell portions. As shown, images are acquired 1001. These images can be from a single frame or multiple frames. As merely an example, an image processing system may analyze such images. One example of

such an image processing system is described below, but should not be construed as limiting certain claims.

In a specific embodiment, cell samples are manipulated using a compound (e.g., substance, drug). The cell samples are imaged for a simple portion or portions, e.g., manipulated cell substructure, manipulated spatial feature of cell, cell density. Image processing techniques are used to extract the feature or features from the image or images. The features can be an independent or a dependent set of cell characteristics (which may be predominately visual) including, for example, count, area, perimeter, length, breadth, fiber length, fiber breadth, shape factor, elliptical form factor, inner radius, outer radius, mean radius, equivalent radius, equivalent sphere volume, equivalent prolate volume, equivalent oblate volume, equivalent sphere surface, average intensity, total intensity, optical density, radial dispersion, texture difference, and others. Each of these features corresponds to a similar manipulation by a compound. Each manipulation forms a new set of features, which are identifiable to the compound. Once each set of features has been extracted, the feature set is populated into a database. Accordingly, the database includes many sets of features, where each set corresponds to a different manipulation for a selected cell. Each set of features corresponding to a manipulation provides a descriptor, which is also stored in the database. The descriptor is a "finger print" including each feature for the manipulation. Each descriptor may be unique, or may have similarities to other descriptors or may even be the same as other descriptors for known and unknown manipulations.

The present system retrieves features, which we define as simple features herein, and forms composite features from them. More than one feature can be combined in a variety of different ways to form these composite features. In particular, the composite feature can be any function or combination of a simple feature and other composite features. The function can be algebraic, logical, sinusoidal, logarithmic, linear, hyperbolic, statistical, and the like. Alternatively, more than one simple feature can be combined in a functional manner (e.g., arithmetic, algebraic). As merely an example, the composite feature equals a sum of feature 1 and feature 2, where these features correspond to the same manipulation. Alternatively, the composite feature equals feature 1 divided by feature 2. Alternatively, the composite feature equals feature 1 minus feature 2. Alternatively,

the composite feature equals a constant times feature 1 plus feature 2. Of course, there are many ways that the composite feature can be defined. The present system also stores 1017 these features in the database. The composite features can also be further combined with simple features. Once these features are defined as descriptors, they are stored 1019 in the database.

Fig. 1B is a simplified diagram of a database system engine 2000 according to an embodiment of the present invention. This diagram is merely an example and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize many other variations, modifications, and alternatives. The engine can be implemented into the present database for populating, searching, and predicting compound or cell characteristics. As merely an example, engine 2001 includes an input/output module 2008. The input/output module is used to input and output information from the database. The information includes, among others, a plurality of feature sets, which correspond to many manipulations. Additionally, the information includes descriptors, which each corresponds to a set of features from the manipulation. The database also has a population module, which is used to configure the features based upon an entity relationship, which has been predetermined.

The database engine also has other modules. In particular, the database has a transcription module, which transfers a preselected set of features and creates a descriptor from them. The transcription module can be used to take a known compound, which has features, to transcribe them into a descriptor. Alternatively, the transcription module can be used to take an unknown compound, which has features, to transcribe them into a descriptor. These descriptors are provided into the database for subsequent use. Finally, the database engine has a prediction module, which can be used to potentially predict a property (e.g., mechanism of action) of an unknown compound. Here, the unknown compound is provided with a descriptor, but the property of the compound is unknown. In one embodiment, the prediction module compares a descriptor of an unknown compound with the many descriptors of known compounds, which were in the populated database. Depending upon the matching criteria, the prediction module will attempt to uncover one or more descriptors of known compounds. Once the prediction module finds the descriptors of the known compounds based upon the descriptor for the unknown compound, it identifies a potential property of such unknown compound for analysis and review. Here, it is

believed that certain features of the known compound, which are similar to those features of the unknown compound may uncover a property to the unknown compound. Details of the present software engine are described more fully below.

Fig. 2 is a simplified block diagram 20 of a cellular knowledge-based system according to an alternative embodiment of the present invention. This diagram is merely an example and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. Like reference numerals are used in the present diagram as the previous diagram for easy cross-referencing, but are not intended to be limiting in any manner. The present diagram 20 includes a variety of elements such as a processor 13 or computing device coupled to a database 11. The processor can be used for retrieving and storing information from the database. The system also includes a plurality of system elements, such as a cleaner 23, a dispenser 25, and an image capturing system 27, which are also coupled to the database in some embodiments. These elements can be coupled to each other through a network or the like. As merely an example, the network can be a NetWareTM network from Novell Corporation or an internet network or the Internet but can also be others and any combination thereof. The system also has an output device 31, which can be used to output information from the database, processor, or other system elements. Details of these elements are described more fully below in reference to the Figs.

Figs. 3-5 are simplified drawings of system elements according to embodiments of the present invention. These diagrams are merely examples and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. As merely an example, Fig. 3 is a simplified diagram of a processor or computing device 13. The computing device 13 includes a bus 112 which interconnects major subsystems such as a central processor 114, a system memory 116 (e.g., random access memory), an input/output ("I/O") controller 118, an external device such as a display screen 124 via a display adapter 126, a keyboard 132 and a mouse 146 via an I/O controller 118, a SCSI host adapter (not shown), and a floppy disk drive 136 operative to receive a floppy disk 138.

The computing device has other features. Storage Interface 134 may act as a storage interface to a fixed disk drive 144 or a CD-ROM player 140 operative

to receive a CD-ROM 142. Fixed disk 144 may be a part of computing device or may be separate and accessed through other interface systems. A network interface 148 may provide a direct connection to a remote server via a telephone link or to the Internet. Network interface 148 may also connect to a local area network ("LAN") or other network interconnecting many computer systems. Many other devices or subsystems (not shown) may be connected in a similar manner. Also, it is not necessary for all of the devices shown in Fig. 3 to be present to practice the present invention, as discussed below. The devices and subsystems may be interconnected in different ways from that shown in Fig. 3. The operation of a computer system such as that shown in Fig. 3 is readily known in the art and is not discussed in detail in this application. Computer code to implement the present invention, may be operably disposed or stored in computer-readable storage media such as system memory 116, fixed disk 144, CD-ROM 140, or floppy disk 138. The computer code can be organized in terms of processes or modules, depending upon the application. That is, the computer code can include a prediction module, a translation module, or other modules to carryout the functionality described herein, as well as others.

Figs. 4 and 5 are simplified diagrams of an imaging system 200 according to an embodiment of the present invention. As shown, the imaging system 200 includes a variety of features such as housing 203, which holds a stage assembly 204. The stage assembly includes an x-stage movement element 206, which is along an x-direction, and a y-stage movement element 207, which is along a y-direction. The imaging system also includes a z-direction movement element, which is perpendicular to the x-y plane. The z-direction movement motor can be attached to the stage, or to the objective nosepiece by way of the microscope housing, or as an external motor between the objective and the microscope housing. The stage can align in any one of the directions to an accuracy of one micron and less, or one-half micron and less, or one-quarter micron and less, depending upon the embodiment.

The stage holds a plate 202 or cell holder, which houses one of a plurality of samples. The plate includes a spatial array 209 of process sites. Each of the process sites can include a plurality of cells and solutions depending upon the embodiment. Each of the sites can carry a sufficient amount of solution to prevent substantial evaporation of the sample during processing in some embodiments. In embodiments for large scale analysis, the plate includes at least 96 sites, or more than

or equal to 384 sites, or more than or equal to 1,536 sites. The plate bottom is transparent and thin, which allows light to pass through the sample. Additionally, the plate is made of a suitable chemical resistant material. As merely an example, the plate can be either a 96, or 384, or 1536 or other formats from places such as Becton Dickinson of Franklin Lakes, NJ, or Corning Science Products of Corning, NY. In a preferred embodiment, the plate is a Corning Costar black-walled 96 well plate catalog #3904 from Corning Science Products of Corning, NY, but should not be limited to these in some applications, but can be others.

Also shown is the condenser for the microscope 201, which can be used to collect phase, DIC, or bright field images of the cells. Images resulting from the illumination of the samples to fluorescence, phase, DIC, or bright field techniques are collected using an image capturing device 208, which captures an image or images of cells from the plate. In a specific embodiment, the microscope is an inverted configuration with the objectives on the bottom of the plate and the condenser disposed overlying an upper surface of the sites, while the image capturing device underlies the sites. Images captured by the imaging device, whether analogue or digital, are viewed by a monitor or other devices. The image capturing device can be any camera assembly such as a charge coupled device camera, which is known as a CCD camera, or other high resolution camera capable of capturing images from the sites. In a specific embodiment, the camera is an interline CCD camera which does not require an external shutter.

In a specific embodiment, the present imaging system can be any suitable unit that is flexible for automated image collection using multi-well plastic plates. The imaging system also should be adapted to collect high-resolution images of cells on plastic or glass plates, cell growth chambers, or coverslips. The system also can be used for imaging multiple cell markers in multiple imaging conditions. To accomplish this, the microscope system has a variety of elements such as a light source, a motorized excitation filter wheel and shutter, x-y-z-motorized stage, excitation and emission filters, Fluor phase and DIC objectives, motorized objective nosepiece, dichroic filters, motorized dichroic filter cubes, phase and DIC rings and prisms, CCD camera, and software control. As merely an example, the present imaging system can have components such as those listed in the Table below.

DESCRIPTION	MAKER	MODEL
Microscope	Zeiss	100M
(x-y) motorized stage	Prior	
Xenon lamp	Sutter	Lambda
Filter wheel	Sutter	Lambda-10
Microtitre Plate holder	Prior	500-H223R
Isolation Table	Kinetic Systems	9101-24-85
Objective Spacers	Polytec PI	P-721.90
Camera	Hamamatsu	C47-95
Computer	IBM	IntelliStation
Software	Metamorph	v.4
Objectives	Zeiss	Achroplan 10x/0.25 LD-Achroplan 20x/0.4 LD-Achroplan 40x/0.6

Table: Image Acquisition System Elements

5 In a specific embodiment, the present system has the following capabilities, which are not intended to be limiting.

Image acquisition

1) Ability to automatically acquire multi-wavelength images from multiple sites on one multi-well plate, to sequentially name image files, and to log any
10 imaging parameter information with image files.

2) Ability to link images with a larger database/spreadsheet of information.

3) Ability to automatically collect multiple plates by interfacing the imaging system with a robotic arm.

15

X-Y control

1) Ability to place 96, 384, or 1536 well plates onto microscope stage and move to each well sequentially.

2) Ability to return to each well and collect another round of images (multi-site time-lapse) or ability to collect rapid time-lapse information at each well (time-lapse of many wells).

3) Ability to collect a low magnification image, automatically
5 determine features which may be of interest, automatically change the objective to a higher magnification, and collect high magnification images of a fixed number of those identified cells in the sample.

4) Ability to collect multiple frames in each site.

10 Z control

1. Ability to auto-focus with substantially minimal damage to biological specimen or fluorophore.

2. Ability to auto-focus rapidly.

15 The present embodiment of the imaging system is shown by way of Figs. 5A and 5B. These diagrams are merely examples and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. The present imaging system 40 includes a variety of elements such as a microscope 41, which is preferably an epi-fluorescent microscope,
20 but can be confocal, multiphoton, or hybrid types. The microscope includes elements 41A, the motorized Z-axis; 41B, the motorized dichroic filter cube holder; and 41C, the motorized objective nosepiece. In one embodiment, the microscope is a Model 100M made by Zeiss. The microscope communicates to computer 51 through control lines 73, 75, and 76. The imaging system also has camera 50 coupled to controller
25 50A and computing device 51, which oversees and controls operations of the elements of the imaging system.

The present microscope includes drivers for spatially moving a stage in two dimensions, including an x-direction, a y-direction, and moving the objective nosepiece in a z-direction in a Cartesian coordinate system. The z-direction
30 movement is provided using a fast z-motor, which can make z-direction adjustments within a predetermined time. The z-direction movement generally provides for focussing of the sample to the camera. The focussing occurs within the predetermined time of preferably ten seconds and less, or five seconds and less, or one

second and less, depending upon the embodiment. As merely an example, the z-motor or positioner can be a model PIFOC objective nanopositioner made by a company called Physik Instrumente of Waldbronn, Germany, but also can be others. The z-motor couples to computer 51 through line 63, which may also include a
5 controller. Depending upon the embodiment, a second z-motor 41A connected to the computer 51 by line 73 may be used to keep the z-motor 42 in the center of its travel. Alternatively, in other embodiments the stage could be provided with a z-motor allowing for movement of the stage in the z-direction.

The present stage also includes an x-y stage 43. The x-y stage moves
10 plate 59, e.g., 96 site, 384 site, 1536 site. The x-y stage moves plate in an x-y spatial manner. The stage has an accuracy or repeatability of about 1 micron and less, or about 2 microns and less. The stage can move in a continuous manner or a stepped manner. The stage also can move up to 30 mm/sec. or faster. The stage also can move 1 mm/sec. and less, depending upon the embodiment. The stage can also step
15 0.1 micron and less or 1 micron and less, as well as other spatial dimensions. The stage can be one such as a Proscan Series made by Prior Scientific of Rockland, MA but can also be others. The stage is controlled via control line 61 through controller 43A, which couples to computer 51 through control line 65.

The stage includes plate holder 44. The plate holder can hold a single
20 plate. In other embodiments, plate holder can also hold multiple plates. The plate holder can use mechanical, electrical, fluid, vacuum and other means for holding the plate or plates. The plate holder also is sufficiently stable for securing the plate. As merely an example, the plate holder is a Model 500-H223R made by Prior Scientific of Rockland, MA. In some embodiments, the plate holder may need adjustment in
25 the z-direction to provide for a desirable focus of a sample on a plate. In these embodiments, the plate holder is supported by spacers 45 or a plurality of stage pins, which mechanically elevate the plate holder in the z-direction. These pins are generally made of a suitable material for supporting such plate holder and also are sufficiently resistant to chemicals and the like.

30 In some embodiments, the entire imaging system is placed on an isolation table 49. The isolation table is disposed between the microscope and support structure. The isolation table is designed to prevent excessive vibration of the plate. The isolation table is made of a suitable material such as steel and honeycomb but can

be others. The table has a thickness of about 8 inches or preferably less than about 24 inches. In one embodiment, the table is Model 9101-24-85 made by Kinetic Systems of Boston, MA.

The imaging system also has a lamp or illumination assembly 62. The lamp assembly provides for a light source (See reference letter B) to a plurality of elements in the imaging system. For easy reading, the light path is defined by the dotted lines, which are not intended to be limiting. The lamp assembly has a variety of elements such as a Xenon lamp 46. The Xenon lamp provides light at about 320 to 700 nanometers (Prefocused). The Xenon lamp is 175 or 300 Watts. As merely an example, the lamp can be a Lambda Model made by Sutter Instrument Company of Novato, CA.

Referring to Fig. 5B, the lamp assembly also has a cold mirror 58, an excitation filter wheel 48, excitation filter(s) 55, and an excitation light shutter 57. As shown, light is derived from the Xenon lamp, reflects off of the cold mirror 58, traverses through the excitation filter or filters 55, and is controlled by the excitation light shutter 57. The lamp assembly has filter wheel 48, which houses one of a plurality of filters, including excitation filters. The shutter and filter wheel are controlled via control lines 67, which are coupled to a computer 51 or other type of computing device. The control lines 67 are coupled through controller 57A (for element 57) and controller 48A (for element 48) via control line 69 to computer 51.

Preferably, light traverses from the lamp assembly through a light guide 47 to illuminate features within the plate. The light guide is suitably selected to have a flexible member, which can be used to place lamp source at a remote location away from the imaging device. The flexible member substantially keeps any vibration from the lamp assembly away from the imaging device. In some embodiments, the member is at least 1 foot away from the imaging device. The light guide is a guide, which is a flexible hose-type sleeve. The sleeve is filled with a liquid such as an aqueous solution containing chloride or phosphate. A thin layer may be formed on the inside of the sleeve. The layer can be a containing tetrafluoroethylene and hexafluoropropylene, or containing tetrafluoroethylene and perfluoromethyl vinyl ether, or tetrafluoroethylene and perfluoropropyl vinyl ether. An example of such a light guide is described in International Application No. WO/98/38537 filed February 29, 1997, and assigned to NATH, Gunther. The liquid

light guide has less than about 30% transmission loss of the light at a remote location such as the imaging system.

Light is derived from the lamp assembly and directs off of filter 56, which directs the light upward. Filter 56 can be a dichroic and emission filter, as well as others. The light traverses through microscope nosepiece 41C, and traverses
5 through objective spacers 54. An objective 53 magnifies the light toward a predetermined point on the plate 59. The objective can be, for example, made by Zeiss of Jena, Germany, as well as other companies. The objective can be one of a plurality including 1X, 10X, 20X, 40X, and others, depending upon the application.
10 Magnification can be further expanded or contracted by intermediate optics between the objective and the camera. Selection of filter or filters is controlled by computer 51 via control line 75.

The camera 50 captures an image of cells from plate 59. The image is obtained from light scattering off of cells or portions of cells in the plate through
15 objective 53, through objective spacers, through filters 56, which are captured at camera 50. In this preferred embodiment, the camera is a digital camera, but can be an analogue camera. The digital camera is a CCD camera, which has 1280 by 1024 pixels, or more or less. The pixels can be 6.7 microns in dimension or more or less. The camera preferably is substantially free from an external shutter to quickly capture
20 a plurality of images of cells from the plate. The camera is controlled via control line 71 through controller 50A, which connects to computer 51 through control line 70. The present invention can also include other types of image acquisition devices selected from at least an epifluorescence, a confocal, a total-internal reflection, a phase, a Hoffman, a bright field, a dark field, a differential interference contrast, an
25 interference reflection, or multi-photon illumination device.

The present imaging system stores images on a high density memory device 60. The high density memory device is preferably optical, but can also be magnetic. The high density memory device can be any suitable unit that is capable of storing a plurality of images from a plurality of sites in the plate. The memory device
30 can be a compact disk, which would generally use a compact disk burner or the like. Depending upon the embodiment, the high density memory device is used to archive the images that are captured from the camera in the imaging system. Further details

of the imaging system can be found throughout the present specification, and more particularly below.

As merely an example, the present invention can be implemented using the following sequence of steps, which have been described in a journal entry form.

- 5 Here, images are opened and objects are identified based on a background value that has been edited in starting image acquisition. Information is maintained in a spreadsheet or other database format, which has the following information for each object:

Image Name	Image Plane	Image Date and Time
Elapsed Time	Object #	Total area
Pixel area	Area	Hole area
Relative hole area	Standard area count	Perimeter
Length	Breadth	Fiber length
Fiber breadth	Shape factor	Ell. form factor
Inner radius	Outer radius	Mean radius
Average gray value	Total gray value	Optical density
Radial dispersion	Texture Difference Moment	EFA Harmonic 2, Semi-Major Axis
EFA Harmonic 2, Semi-Minor Axis	EFA Harmonic 2, Semi-Major Axis Angle	EFA Harmonic 2, Ellipse Area
EFA Harmonic 2, Axial Ratio	EFA Harmonic 3, Semi-Minor Axis	

10

After computations are done, the log file is saved. In particular, the file is saved in an appropriate place with an appropriate name.

In a specific embodiment, the present invention provides the following detailed example of journal entries, which should not limit the scope of the invention.

Set Up Sequential File Names	Interactive: user sets up prefix name and image storage directory
Open Data Log	Opens a DDE (Excel) File
Annotate Log File	Interactive: experimental information that will go into the first line of the log file of stage positions
Stage (Go to Origin)	Origin is set as the center of well A1
Stage (Move to Absolute Position)	Offset to upper left hand corner of well (1410, 1621)
Stage (Log Position)	
Stage (Scan Wells)	User picks wells to scan: runs 3x3 image collection.jnl.

3X3 IMAGE COLLECTION.jnl

Stage (Scan)	Takes 9 images of well, -1600 motor steps apart from left to right 3 columns and 3 rows, runs FOCUS, COLLECT IMAGE, SAVE SEQUENTIAL FILE NAME.JNL.
--------------	--

5

FOCUS, COLLECT IMAGE, SAVE SEQUENTIAL FILE NAME.jnl

Stage (Log Position)	Logs stage position of each image
ADC – Focus	Opens up the manual focusing window with whatever focus time is current set
Show Message and Wait	Interactive: user hits enter to continue when done focusing

ADC-Acquire from Digital Camera	Takes Hoechst image
Save Using Sequential File Names	
Close	Closes image window

START IMAGE ANALYSIS.jnl

Low Pass	3x3 convolution of already opened image
Low Pass	3x3
Show Region Statistics	Interactive: Show entire image statistics. Calculate background subtraction value for step 4. by: INTENSITY Average + INTENSITY Std. Dev.
Arithmetic	Interactive: User inputs subtraction value from 3. into the constant Value field
Threshold image	Creates threshold 1 unit above 0 to 4096
Integrated Morphometry – Load State	Loads Start Image Analysis.ima Classifier 100 < area < 200000
Integrated Morphometry – Measure	Interactive: Shows area summary information about all objects. The average number is used as the Standard Area in 8.
Object Standards - Set Object Standards	Interactive: User inputs average area value from 7. into Standard Area box to be used by automated IMA for all images

IMA OBJECTS.jnl

Low Pass	3x3 convolution
Low Pass	3x3 convolution
Arithmetic	This background subtraction value needs to be manually entered into this journal from the value determined in START IMAGE ANALYSIS.jnl step 3
Threshold Image	1 unit above 0
Integrated Morphometry – Load State	Hoechst. IMA Classifier 200 < area < 200000
Integrated Morphometry – Measure	Measures statistical info for all objects
Run Journal	Runs log obj and sum data.jnl

Log obj and sum data.jnl

Integrated Morphometry – Log Data	Logs object data into Sheet 1
Integrated Morphometry – Log Data	Log summary data into Sheet 2

5

COLLECT AUTOMATED IMA DATA IN ONE SPREADSHEET.jnl

Run Journal	Runs OPEN OBJECT LOG DDE FILE.JNL
Loop for all Images in a Directory	Loops IMA OBJECTS.jnl
Close Summary Log	
Close Object Log	User must manually save Excel spreadsheet

OPEN OBJECT LOG DDE FILE.jnl

Open Object Log	Opens a DDE object log into sheet 1 of an Excel spreadsheet
Open Summary Log	Opens a summary log into sheet 2

COLLECT AUTOMATED IMA DATA IN ONE SPREADSHEET 16 BIT IMAGES.jnl

Arithmetic	Interactive: Opens Arithmetic window for user to input background subtraction level from START IMAGE ANALYSIS.jnl step 3
Run Journal	Runs OPEN OBJECT LOG DDE FILE.JNL
Loop for all Images in a Directory	Interactive: Runs IMA OBJECTS 16 bit.jnl. User picks directory from which to choose.

5

IMA OBJECTS 16bit.jnl

Low Pass	3x3 convolution
Low Pass	3x3 convolution
Copy to 8-bit Image	No autoscale, to new untitled image
Save Using Sequential File Name	Saves 8bit image using previously defined Sequential File names.
Arithmetic	This background subtraction value needs to be manually entered into this journal from the value determined in START IMAGE ANALYSIS16 TO 8 BIT.jnl step 5
Threshold Image	1 unit above 0 to 255

Integrated Morphometry – Load State	Hoecsht.IMA Classifier 200 < area < 200000
Integrated Morphometry – Measure	Measures statistical info for all objects
Run Journal	Runs log obj and sum data.jnl

START IMAGE ANALYSIS 16 to 8 BIT.jnl

Copy to 8-bit Image	No autoscale, to new untitled image
Close	Closes 16 bit image
Low Pass	3x3 convolution
Low Pass	3x3 convolution
Show Region Statistics	Interactive: Show entire image statistics. Calculate background subtraction value for step 6. by: INTENSITY Average + INTENSITY Std. Dev.
Arithmetic	Interactive: User inputs subtraction value from 5. into the constant Value field
Threshold image	Creates threshold by 1 unit above 0 to 255
Integrated Morphometry – Load State	Loads Start Image Analysis.ima Classifier 100 < area < 200000
Integrated Morphometry – Measure	Interactive: Shows area summary information about all objects. The average number is used as the Standard Area in 10.
Object Standards - Set Object Standards	Interactive: User inputs average area value from 9. into Standard Area box to be used by automated IMA for all images

IMA OBJECTS WITH NEW LOG FILE.jnl

Run Journal	OPEN OBJECT LOG DDE FILE.JNL
Run Journal	IMA OBJECTS.jnl
Close Summary Log	
Close Object Log	User must manually save every Excel spreadsheet generated.

INTERACTIVE IMA OBJECTS.jnl

Threshold Image	User manually sets threshold
Integrated Morphometry – Load State	Hoechst.IMA Classifier $200 < \text{area} < 200000$
Integrated Morphometry – Measure	Objects
Integrated Morphometry – Log Data	Into open object.log file

5

COLLECT INTERACTIVE IMA DATA.jnl

Close Object Log	
Open Object Log	Interactive
Annotate Log File	Interactive: experimental information that will go into the first line of the object log file
Loop for all Images in Directory	Runs INTERACTIVE IMA OBJECTS.jnl

CHANGE FILTER, COLLECT IMAGE, SAVE SEQUENTIAL FILE
NAME.jnl

Stage (Log Position)	
ADC-Focus	

Show Message and Wait	Interactive – user presses Enter when done focusing
ADC – Acquire from Digital Camera	Hoechst
Save Using Sequential File Name	
Close	Close open image

COLLECT HOECHST AND FITC.jnl

Run Journal	FOCUS, COLLECT IMAGE, SAVE SEQUENTIAL FILE NAME.JNL
Run Journal	CHANGE FILTER, COLLECT IMAGE, SAVE SEQUENTIAL FILE NAME.jnl

3X3 IMAGE COLLECTION HOECHST FITC.jnl

Stage (Scan)	COLLECT HOECHST AND FITC.jnl
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5

AUTOMATED 3X3 IMAGE COLLECTION HOECHST FITC.jnl

Set Up Sequential File Names	Interactive: user sets up prefix name and image storage directory
Open Data Log	Excel DDL files
Annotate Log File	Interactive: experimental information that will go into the first line of the log file of stage positions
Stage (Go to Origin)	Origin is set as the center of well A1
Stage (Move to Absolute Position)	Offset to upper left hand corner of well (1410, 1621)

Stage (Log Position)	
Stage (Scan Wells)	Interactive: user picks wells to scan: runs 3X3 IMAGE COLLECTION HOECHST FITC.jnl

AUTOMATED IMAGE COLLECTION.jnl

Set Up Sequential File Names	Interactive: user sets up prefix name and image storage directory
Open Data Log	Opens a DDE (Excel) File
Annotate Log File	Interactive: experimental information that will go into the first line of the log file of stage positions
Stage (Go to Origin)	Origin is set as the center of well A1
Stage (Log Position)	
Stage (Scan Wells)	Interactive: user picks wells to scan: runs FOCUS, COLLECT IMAGE, SAVE SEQUENTIAL FILE NAME.JNL. Well to well travel = (-9035, -9035)

5

STARTUP.jnl

Install and Configure Devices	Open Stage Meta Devices
Set Live Video Channel	

Preferences	<u>Measure Objects</u> : Draw failed classifier objects, Exclude objects that touch the edge of the image, Enable Elliptical Fourier Parameters, turn off Warn users when measurement data will be erased <u>Image Saving</u> : Save Tiff/stk using LZW compression <u>Image Windows</u> : Use transparent thresholds.
Configure Default Paths	C:\Metamorph Data C:\Metamorph Data\Common Settings
Load Journal Taskbar	Common.JTB

Nested Journals

Automated 3x3 Image Collection

- 5 *Loop* 3x3 image collection
 Loop focus, collect image, save sequential file name

Automated 3x3 image collection Hoechst FITC

- 10 *Loop* 3x3 image collection Hoechst FITC
 loop Collect Hoechst and FITC
 focus, collect image, save sequential file name
 change filter, collect image, save sequential file name

Automated image collection

- 15 *Loop* focus, collect image, save sequential file name

Collect automated IMA data in one Spreadsheet

Open object log DDE file

Loop IMA objects

Log obj and sum data

Collect automated IMA data in one spreadsheet 16 bit images

5 Open object log DDE file

Loop IMA objects 16 bit

Log obj and sum data

Although the above has been generally described in terms of a specific
10 user interface and software code, other user interfaces and code can also be used. One
of ordinary skill in the art would recognize many other variations, alternatives, and
modifications.

Fig. 6 is a simplified diagram 600 of a cleaning and dispensing system
according to an embodiment of the present invention. This system 600 includes a
15 variety of elements such as a dispensing head 609, which is coupled to a plurality of
pipettes 601. The pipettes input and output fluids or solutions from plate 603. The
plate has a plurality of sites, each of which can be used to input cells or a combination
of cells and solution. The system also has elements to house solutions 605, which are
used to manipulate cell samples in the plate. The dispensing head is supported
20 through a support member 607, which is sufficiently rigid to allow for movement of
the head. The dispenser is coupled to the present system in a mechanical and
electrical manner, which provides for a fully integrated system for providing cell
samples to the imaging system according to the present invention.

Fig. 7A illustrates a representative block flow diagram of simplified
25 process steps of a method for determining properties of a manipulation based upon
effects of the manipulation on one or more portions of one or more cells in a
particular embodiment according to the present invention. This diagram is merely an
illustration and should not limit the scope of the claims herein. One of ordinary skill
in the art would recognize other variations, modifications, and alternatives. In step
30 700, one or more samples of cells can be provided. These cells can be live, dead, or
fixed cells, or cell fractions. The cells also can be in one of many cell cycle stages,
including G0, G1, S, G2 or M phase, M phase including the following cell cycle
stages: interphase, prophase, prometaphase, metaphase, anaphase, and telophase.

Cell components tracked in presently preferable embodiments can include proteins, protein modifications, genetically manipulated proteins, exogenous proteins, enzymatic activities, nucleic acids, lipids, carbohydrates, organic and inorganic ion concentrations, sub-cellular structures, organelles, plasma membrane, adhesion complex, ion channels, ion pumps, integral membrane proteins, cell surface receptors, G-protein coupled receptors, tyrosine kinase receptors, nuclear membrane receptors, ECM binding complexes, endocytotic machinery, exocytotic machinery, lysosomes, peroxisomes, vacuoles, mitochondria, Golgi apparatus, cytoskeletal filament network, endoplasmic reticulum, nuclear membrane, proteosome apparatus, chromatin, nucleolus, cytoplasm, cytoplasmic signaling apparatus, microbe specializations and plant specializations.

The following table illustrates some markers and cell components commonly used by embodiments according to the present invention. Other markers can be used in various embodiments without departing from the scope of the invention.

Cell component	Marker	Disease State
Plasma membrane (including overall cell shape)	Carbocyanine dyes Phosphatidylserine Various lipids Glycoproteins	Apoptosis-Cancer Apoptosis-Neural degenerative Ds
Adhesion complexes	Cadherins Integrins Occludin Gap junction ERM proteins CAMs Catenins Desmosomes	Thrombosis Metastasis Wound healing Inflammatory Ds Dermatologic Ds
Ion Channels and Pumps	Na/K Atpase Calcium channels Serotonin reuptake pump CFTR	Cystic fibrosis Depression Congestive Heart Failure Epilepsy

G coupled receptors	β adrenergic receptor Angiotensin receptor	Hypertension Heart Failure Angina
Tyrosine kinase receptors	PDGF receptor FGF receptor IGF receptor	Cancer Wound healing Angiogenesis Cerebrovascular Ds
ECM binding complexes	Dystroglycan Syndecan	Muscular Dystrophy
Endocytotic machinery	Clathrin Adaptor proteins COPs Presenilins Dynamin	Alzheimer's Ds
Exocytotic machinery	SNAREs Vesicles	Epilepsy Tetanus Systemic Inflammation Allergic Reactions
Lysosomes	Acid phosphatase Transferrin	Viral diseases
Peroxisomes/Vacuoles		Neural degenerative Ds
Mitochondria	Caspases Apoptosis inducing factor F1 ATPase Fluorescein Cyclo-oxygenase	Apoptosis Neural degenerative Ds Mitochondrial Cytopathies Inflammatory Ds
Golgi Apparatus	Lens Culinaris DiOC6 carbocyanine dye COPs	

Cytoskeletal Filament Networks	Microtubules	Cancer
	Actin Intermediate Filaments Kinesin, dynein, myosin Microtubule associated proteins Actin binding proteins Rac/Rho Keratins	Neural degenerative Ds Inflammatory Ds Cardiovascular Ds Skin Ds
Endoplasmic Reticulum	SNARE PDI Ribosomes	Neural degenerative Ds
Nuclear Membrane	Lamins Nuclear Pore Complex	Cancer
Proteosome Apparatus	Ubiquityl transferases	Cancer
Chromatin	DNA Histone proteins Histone deacetylases Telomerases	Cancer Aging
Nucleolus	Phase markers	
Cytoplasm	Intermediary Metabolic Enzymes BRCA1	Cancer
Cytoplasmic Signaling Apparatus	Calcium Camp PKC pH	Cardiovascular Ds Migraine Apoptosis Cancer
Microbe Specializations	Flagella Cilia Cell Wall components: Chitin synthase	Infectious Ds

Plant specializations	Choloroplast	Crop Protection
	Cell Wall components	

Then, in a step 702, one or more samples of the manipulation can be provided to the cells. Manipulations can comprise one or any combination of chemical, biological, mechanical, thermal, electromagnetic, gravitational, nuclear, or temporal factors, for example. For example, manipulations could include exposure to chemical compounds, including compounds of known biological activity such as therapeutics or drugs, or also compounds of unknown biological activity. Or exposure to biologics that may or may not be used as drugs such as hormones, growth factors, antibodies, or extracellular matrix components. Or exposure to biologics such as infective materials such as viruses that may be naturally occurring viruses or viruses engineered to express exogenous genes at various levels. Bioengineered viruses are one example of manipulations via gene transfer. Other means of gene transfer are well known in the art and include but are not limited to electroporation, calcium phosphate precipitation, and lipid-based transfection. Manipulations could also include delivery of antisense polynucleotides by similar means as gene transfection. Other genetic manipulations include gene knock-outs or gene mutations. Manipulations also could include cell fusion. Physical manipulations could include exposing cells to shear stress under different rates of fluid flow, exposure of cells to different temperatures, exposure of cells to vacuum or positive pressure, or exposure of cells to sonication. Manipulations could also include applying centrifugal force. Manipulations could also include changes in gravitational force, including sub-gravitation (the preferred embodiment in outer space). Manipulations could include application of a constant or pulsed electrical current. Manipulations could also include irradiation. Manipulations could also include photobleaching which in some embodiments may include prior addition of a substance that would specifically mark areas to be photobleached by subsequent light exposure. In addition, these types of manipulations may be varied as to time of exposure, or cells could be subjected to multiple manipulations in various combinations and orders of addition. Of course, the type of manipulation used depends upon the application.

Then, in a step 704, one or more descriptors of a state in the portions of the cells in the presence of the manipulation can be determined using the images

collected on the imaging system. Descriptors can comprise scalar or vector values, representing quantities such as area, perimeter, dimensions, intensity, gray level, aspect ratios, and the like. Other types of descriptors include, but are not limited to, one or any combination of characteristics such as a cell count, an area, a perimeter, a
 5 length, a breadth, a fiber length, a fiber breadth, a shape factor, a elliptical form factor, an inner radius, an outer radius, a mean radius, an equivalent radius, an equivalent sphere volume, an equivalent prolate volume, an equivalent oblate volume, an equivalent sphere surface area, an average intensity, a total intensity, and an optical
 10 density. These descriptors can be average or standard deviation values, or frequency statistics from the descriptors collected across a population of cells. These descriptors can be further reduced using other methods such as principal component analysis and the like. In some embodiments, the descriptors include features from different cell portions or cell types. That is, a first feature can be from a nuclei and a second feature is from another cell structure such as Golgi apparatus, mitochondria, spacing between
 15 cell structures or cells themselves, as well as many others.

A presently preferable embodiment uses descriptors selected from the following table. Other descriptors can also be used without departing from the scope of the invention.

Name of Parameter	Explanation/Comments
Count	Number of objects
Area	
Perimeter	
Length	X axis
Width	Y axis
Shape Factor	Measure of roundness of an object
Height	Z axis
Radius	
Distribution of Brightness	
Radius of Dispersion	Measure of how dispersed the marker is from its centroid
Centroid location	x-y position of center of mass
Number of holes in closed objects	Derivatives of this measurement might include, for

	example, Euler number (= number of objects - number of holes)
Elliptical Fourier Analysis (EFA)	Multiple frequencies that describe the shape of a closed object
Wavelet Analysis	As in EFA, but using wavelet transform
Interobject Orientation	Polar Coordinate analysis of relative location
Distribution Interobject Distances	Including statistical characteristics
Spectral Output	Measures the wavelength spectrum of the reporter dye. Includes FRET
Optical density	Absorbance of light
Phase density	Phase shifting of light
Reflection interference	Measure of the distance of the cell membrane from the surface of the substrate
1,2 and 3 dimensional Fourier Analysis	Spatial frequency analysis of non closed objects
1,2 and 3 dimensional Wavelet Analysis	Spatial frequency analysis of non closed objects
Eccentricity	The eccentricity of the ellipse that has the same second moments as the region. A measure of object elongation.
Long axis/Short Axis Length	Another measure of object elongation.
Convex perimeter	Perimeter of the smallest convex polygon surrounding an object
Convex area	Area of the smallest convex polygon surrounding an object
Solidity	Ratio of polygon bounding box area to object area.
Extent	proportion of pixels in the bounding box that are also in the region
Granularity	
Pattern matching	Significance of similarity to reference pattern
Volume measurements	As above, but adding a z axis

Then, in a step 705, a database of cell information can be provided.

Next, in a step 706, a plurality of descriptors can be searched from a database of cell information in order to locate descriptors based upon one of the descriptors of the manipulation. Then, in a step 708, properties of the manipulation are predicted based upon the properties of the located descriptors. Properties can comprise toxicity, specificity against a subset of tumors, mechanisms of chemical activity, mechanisms of biological activity, structure, adverse biological effects, biological pathways, clinical effects, cellular availability, pharmacological availability, pharmacodynamic properties, clinical uses and indications, pharmacological properties, such as absorption, excretion, distribution, metabolism and the like.

In a particular embodiment, step 706 comprises determining matching descriptors in the database corresponding to a prior administration of the manipulation to the descriptors of the present administration of the manipulation. In a particular embodiment according to the present invention, combinations of measurements of scalar values can provide predictive information. A database can be provided having one or more "cellular fingerprints" comprised of descriptors of cell-substance interactions of drugs having known mechanisms of action with cells. Such descriptors can be analyzed, classified, and compared using a plurality of techniques, such as statistical classification and clustering, heuristic classification techniques, a technique of creating "phylogenetic trees" based on various distance measures between descriptors from various drugs. In this embodiment, numeric values for the descriptors can be used by comparison techniques. A phylogenetic tree can be created that illustrates a statistical significance of the similarity between descriptors for the drugs in the database. Because the drugs used to build the initial database are of known mechanism, it can be determined whether a particular scalar value in a descriptor is statistically predictive. Finally, a compound descriptor with no known mechanism of action can be queried against the database and be statistically compared and classified among the drugs in the database that the compound most resembles.

In a particular embodiment, relationships between measured morphological properties of images and physiological conditions can be determined. Relationships can include, for example, treatment of different cell lines with chemical compounds, or comparing cells from a patient with control cells, and the like. In a presently preferable embodiment, comparisons can be performed on acquired image

features. Some embodiments can comprise statistical and neural network - based approaches to perform comparisons of various features. The foregoing is provided as merely an example, and is not intended to limit the scope of the present invention. Other techniques can be included for different types of data.

5 In some embodiments, classification, clustering and other types of predictive data analysis can be performed on features extracted from cell images. In a presently preferable embodiment, statistical procedures for comparisons, classification and clustering are performed on data obtained from imaging cells.

Fragments of data preparation and pre-formatting (S language):

```
10       >tmp.frame <- Generic.Summary  
      >names1 <- paste("Cell.line.5", tmp.names, sep=".")  
      > by.compound.matrix <- as.matrix(arranged.by.compound)
```

Example of the code for principal component analysis (data
15 preparation) using S language:

```
      all.data.princomp <- menuPrincomp(data =  
      by.compound.matrix, scores = T, cor = "Correlation",  
      na.action = T, print.short = T, print.importance = T,  
      print.loadings = T, cutoff.loadings = 0.1,  
20   plot.screepplot = T, plot.loadings = T, plot.biplot = T,  
      plot.biplot.choices = c(1,2), predict.p = F)
```

Example of clustering using a divisive hierarchical clustering
algorithm:

```
25       > div.hier.2.manhattan.cluster$call  
      diana(x = tmp.sum.by.comp, diss = F, metric =  
      "manhattan",  
      stand = T, save.x = T, save.diss = T)
```

30 Another embodiment utilizes existing tools for biological sequence similarity searches, classification, and phylogenetic analysis. In a particular embodiment, numbers in a numerical descriptor can be substituted by one or more of nucleic acid or amino acid codes according to a one of several sets of rules. Once

converted into a corresponding nucleotide or amino acid sequence representation, the fingerprints can be analyzed and compared using software and algorithms known in the art for genetic and peptide sequence comparisons, such as GCG, a product of Genetics Computer Group, with company headquarters in Madison WI. Select
5 embodiments comprising such approaches enable the use of a broad array of sophisticated algorithms to compare, analyze, and cluster gene and protein sequences. Many programs performing this task are known to those of ordinary skill in the art, such as for example, the PHYLIP (PHYlogeny Interference Package) a package of programs for inferring phylogenies (evolutionary trees) described in (Feldenstein, J.
10 1996 Methods Enzymol 266:418-427 and Feldenstein, J. 1981 J. Mol. Evol. 17(6):368-376).

Embodiments can perform such analysis based upon factors such as numerical value, statistical properties, relationships with other values, and the like. Further details of a step of manipulation are noted more particular below.

15 Fig. 7B illustrates a representative block flow diagram of simplified process steps for determining one or more descriptors of a state in the portions of the cells in the presence of the manipulation of step 704 of Fig. 7A in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill
20 in the art would recognize other variations, modifications, and alternatives. In a step 712, an image of a cell portion is obtained. In some embodiments, the cell portion is visualized with a fluorescently labeled marker that is specific for the portion or portions of interest. A cell portion can include, for example, one or more of the following: nuclei, Golgi apparatus, and other features. The cell portion may vary in
25 select embodiments according to the invention. Then, in a step 714, a digitized representation of the image obtained in step 712 is determined. In some embodiments, steps 714 and step 712 can comprise a single step. These embodiments use a digital imaging means such as a digital camera, to obtain a digital image of the target directly. Next, in a step 716, the digital representation of the image is
30 processed to obtain image features. Image features can include such quantities as area, perimeter, dimensions, intensity, aspect ratios, and the like. Then, in a step 718 descriptors can be determined from the image features. Descriptors can comprise scalar or vector quantities and can comprise the image features themselves, as well as

composed features, such as shape factor derived by a relationship $4\pi * \text{area} / \text{perimeter}$, and the like. Descriptors can also comprise statistical quantities relating to feature characteristics across a population of cells, such as a standard deviation, and average, and the like.

5 In a preferred embodiment, cells can be placed onto a microscope, such as a Zeiss microscope, or its equivalent as known in the art. A starting point, named Site A01, is identified to the microscope. A plurality of exposure parameters can be optimized for automated image collection and analysis. The microscope can automatically move to a new well, automatically focus, collect one or more images, at
10 one or more wavelengths, move to a next well, and repeat this process for all designated wells in a multiple well plate and for multiple plates. A file having a size and an intensity distribution measurement for each color and rank for each well can then be created for the images acquired. Based on this information, a user or a computer can revisit sites of interest to collect more data, if desired, or to verify
15 automated analysis. In a presently preferred embodiment, image automatic focus and acquisition can be done using computer software controlling the internal Z-motor of the microscope. Images are taken using a 10x, 20x, or 40x air long working distance objectives. Sometimes multiple images are collected per well. Image exposure times can be optimized for each fluorescent marker and cell line. The same exposure time
20 can be used for each cell line and fluorescent marker to acquire data.

Fig. 7C illustrates a representative block flow diagram of simplified process steps for obtaining images of cell portions of step 712 of Fig. 7B in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill
25 in the art would recognize other variations, modifications, and alternatives. The method is generally outlined by the steps below:

- (1). In a step 720, a sample is provided to the imaging device. Samples can be provided in 96 well plates and the like. The sample may be loaded into a microscope, such as a Zeiss microscope or equivalent.
- 30 (2). In a step 722, a set of optical filters is selected to shine light of the appropriate wavelength to illuminate the first sample, which may be contained in a first well designated A01.

(3). In a step 724, an automatic focusing procedure is performed for the site. In a particular embodiment, the internal z-motor of the microscope which is attached to the objective nosepiece is used for automatic focusing of the microscope. In an alternative embodiments, the plate holding the samples is moved to perform
5 automatic focusing of the microscope, or focusing can be performed by moving optical components attached to the microscope and the like.

(4). In a step 726, images are collected for the site. Images can be collected for every color at every site. Present embodiments can provide images for up to four colors. However, embodiments are contemplated that can provide more
10 colors by using either a monochromator coupled with excitation filters which are on a filter wheel, or by digitally separating overlapping fluorophores. Those knowledgeable in the field will know that given calibration images of single fluorophores, a look-up table can be devised which will allow for the digital removal of fluorescence bleed-through of fluorescence which may occur in optical channels
15 other than the one for which that filter has been optimized in instances of using more than one fluorophore at once. Cell growth and density information is also collected. Cell density is determined by what percentage of the area being imaged is inhabited by cells. In some embodiments, imaging can be facilitated using one or more biosensors, molecules such as non-proteins, i.e., lipids and the like, that are
20 luminescently tagged. However, some embodiments can also use fluorescence polarization and the like. Fluorescence polarization is a homogeneous fluorescence technology where the excited state of the molecule lasts much longer than in normal fluorescence, taking seconds to minutes to reach equilibrium, obliterating the need to wash away fluorescence markers that are not specifically bound to a marker. Further,
25 embodiments can detect differences in spectral shifts of luminescent markers. Some fluorescence markers, such as Nile Red sold by Molecular Probes of Eugene, OR, will change its emission peak wavelength depending on its environment. One can detect these changes by monitoring the level of fluorescence at both wavelengths and reading out at ratio of the two.

30 (5). In a step 728, a determination is made whether more fields of view need to be taken for a particular color. If this is so, then processing continues at step 726 at a new site. Otherwise, processing continues with a decisional step 730.

Images can now be taken by repeating step 726. In a preferred embodiment 4 to 9 images are collected at each site.

(5). In a step 730, a determination is made whether more optical configurations need to be taken in order to obtain images for all differently-marked cell portions the sample. If this is so, then in a step 732 a new optical configuration is determined. Images for the new optical configuration can now be taken by repeating steps 726 and 728.

(6). In a decisional step 734, after all optical configurations and images for fields of view in a sample have been obtained, a determination is made whether any further samples remain to be analyzed. If so, a new sample is brought into view and processing continues with step 720. Otherwise, image processing is complete. In a presently preferable embodiment, image data can be stored on a CD ROM using a CD ROM burner, such as CRW4416 made by Yamaha of Japan. However, other mass storage media can also be used.

Fig. 7D illustrates a representative block flow diagram of simplified process steps for processing digitized representations of step 716 of Fig. 7B in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. The method is generally outlined by the steps below:

(1). In a step 740, a digitized image input is preprocessed. Preprocessing might include, but is not limited to, such operations as background subtraction, thresholding, smoothing, adoptive filtering, edge enhancements, contrast enhancements, histogram equalization. A particular combination of preprocessing steps can be applied to images in successive steps or in parallel to copies of the image.

A simplified example of a smoothing and background subtraction procedure in a MatLab language is presented in computer code below:

```
function Isubtracted = cmBackgrSubtr1(I,k)
% cmBackgrSubtr1(I,k) - simple flat background (=modal*k)
subtraction
% Y = cmBackgrSubtr1(I, k) - image Y is generated by
```



```

    % subtraction (with saturation) of modal pixel value of I
    multiplied by k
    % DEFAULT - k=1
    %
5   if (nargin == 1)
        k=1;
    end
    if (size(k)~=1)
        error('cmBackgrSubtrl: parameter k should be a number.
10   Exiting...');
    end

    %modpixnum = floor(size(I(:),1)/2);
    %sortedval = sort( double(I(:)) );
15  %modpixel = sortedval(modpixnum);
    modpixel = median(double(I(:)));
    bg = k*modpixel;

    Isubtracted = mmsubm( uint8(I), uint8(round(ones(
20  size(I))*k*modpixel )) );

```

An example of a procedure for thresholding in computer code (MatLab) is presented below:

```

function thresh = GetThreshByPerim1(I, M)
25  % GetThreshByPerim1(I) Finds optimal thresholding value
    for image I
    % N = GetThreshByPerim1(I) Finds thresholding value N for
    image I
    % N = GetThreshByPerim1(I, M) - tests threshold values up
30  to M
    % DEFAULT M = maximum pixel value in I
    % note that GetThreshByArea is significantly faster
    % finds a threshold value that causes the maximal change
    in the

```

```
% total perimeter of the objects (Russ ????)
% see Matlab_Auto_threshold1_1-23-99.doc for more details
% Note: works somewhat better on SMOOTH images (i.e.
medfilt2(I, [3 3]) two times

5
if (nargin == 0)
    error (strcat( mfilename, ' : at least one parameter
required')));
elseif (nargin == 1)
10    M = double(max(I(:)));      %test thresholds up to
    maximum pixel value in I
elseif (nargin > 2)
    error (strcat (mfilename, ' : too many parameters'));
end

15
if (size(M)>1)
    error (strcat(mfilename, ' : argument M should be a
number')));
end

20
Minval = double( min(I(:)));
step = 1;

%generate vertical vector perims with total perimeters of
25 objects at different
%threshold values
for i=Minval : step : M
    bwI = im2bw(I, i/255);
    prI = bwperim(bwI);
30    pr = sum(prI(:));
    if (exist('perims', 'var') == 0) %perims is yet
undefined
        perims = pr;
    else
```

```

        perims = cat(1, perims, pr);
    end
end

5  % vector prdiffs contains differences between successive
    perimeters
    prdiffs = diff(perims);
    mindecrease = min(prdiffs);
    minvalues = find(prdiffs == mindecrease);
10  index_of_mindecrease = minvalues(1);
    thresh = index_of_mindecrease + 1;

    % =====end GetThresh1=====

```

15 Thresholding provides a specific intensity, such that pixels darker than the threshold are deemed black, and pixels lighter than the threshold are considered white. The thresholded image can be processed using binary image processing techniques in order to extract regions.

(2). In a step 742+744, the digitized image input is subjected to object
20 identification. This can be accomplished by a variety of procedures, for example by thresholding or edge detection and subsequent morphological opening and closing. Edge detection can be accomplished by means of gradient-based or zero-crossing methods, such as Sobel, Canny, Laplassian, Perwitt, and other methods.

An example of object identification procedure based on Canny edge
25 detection (in MatLab language) is presented below:

```

function Imask = cmMaskDNA1(I);
% cmMaskDNA1 - generates binary mask for cell nuclei
through edge detection
30 % Imask = cmMaskDNA1(I)
% PARAMETERS
%   I - intensity image (grayscale)
% OUTPUT
%   Imask - BW image with objects from I

```

```

%
% For more details see Notebook Matlab_DNA_masking1_1-22-
99.doc
% Uses SDC Morphology Toolbox V0.7

5
if (nargin ~= 1)
    error('Wrong number of input parameters');
end
if (nargout ~= 1)
10    error('Wrong number of output parameters: one output
argument should be provided');
end

15    Imask = edge(I, 'canny');
    Imask = mm dil(Imask, mmsecross(1));
    Imask = mmero ( mmc lohole(Imask, mmsecross(1)));
    Imask = mmedgeoff(Imask, mmsecross(1));
    % note that mmedgeoff this command removed FILLED OBJECTS
20    but not touching OUTLINES.
    % these outlines can be removed by filtering:
    Imask = medfilt2(Imask, [5 5]);

    %=====end cmMaskDNA1
25    =====

```

However, embodiments can also use other techniques, such as Fast Fourier Transforms (FFT) and the like as known in the art without departing from the scope of the present invention.

30 (3). In a step 746, a plurality of region features can be determined. For example, in a representative embodiment, image features can include such quantities as area, perimeter, dimensions, intensity, aspect ratios, and the like. Features not directly related to individual objects are also being extracted.

An example of a procedure for extraction of some of the features (MatLab language) is presented below:

```

function OData = cmGetObjectsData(I, Ilabel)
5  % cmGetObjectsData returns array measurements of objects
  in image "I" masked by "Ilabel"
  % EV 2-3-99; 2-10-99
  % OData = cmGetObjectsData(I, Ilabel) returns an array of
  morphological and intensity measurements
10  %   taken from a grayscale image "I". Objects are
  identified on a mask image Ilabel, usually
  %   created by bwlabel()
  % OUTPUT:
  % Each row in the output array OData represents
15  individual object
  % columns contain the following measurements:
  %
  %   1 - Index ("number" of an object);      8 -
  Solidity;
20  %   2 - X coordinate of the center of mass; 9 - Extent;
  %   3 - Y coordinate      "-"; 10 - Total
  Intensity;
  %   4 - Total Area (in pixels);              11 - Avg.
  Intensity;
25  %   5 - Ratio of MajorAxis/MinorAxis;      12 - Median
  Intensity;
  %   6 - Eccentricity;                        13 - Intensity of
  20% bright pixel
  %   7 - EquivDiameter;                       14 - Intensity of
30  80% bright pixel
  %
  % For details on morphological parameters see information
  on MatLab imfeature();

```

```
% Intensity parameters are either obvious or are
documented in comments in this file.

if (nargin ~= 2)
5   error ('function requires exactly 2 parameters');
end
if (nargout ~= 1)
    error ('function has 1 output argument (array X by
14) ');
10 end

% finished checking arguments

% first collect morphological parameters in a structure
15 array:
ImStats = imfeature(Ilabel, 'Area', 'Centroid',
    'MajorAxisLength',...
        'MinorAxisLength', 'Eccentricity', 'EquivDiameter',
    ...
20    'Solidity', 'Extent', 8 );

% now convert it into array (matrix) while collecting
intensity data for each object:

25 %preallocate output array:
numobjects = size(ImStats, 1);
OData = zeros(numobjects, 14);
%now convert ImStats into array and add intensity data to
it
30 for k=1:numobjects
    OData(k, 1) = k;
    OData(k, 2) = ImStats(k).Centroid(1);
    OData(k, 3) = ImStats(k).Centroid(2);
    OData(k, 4) = ImStats(k).Area;
```

```

        OData(k, 5) = (ImStats(k).MajorAxisLength) /
        (ImStats(k).MinorAxisLength);
        OData(k, 6) = ImStats(k).Eccentricity ;
        OData(k, 7) = ImStats(k).EquivDiameter;
5      OData(k, 8) = ImStats(k).Solidity;
        OData(k, 9) = ImStats(k).Extent;

        % now collect and assign intensity parameters from
        image I
10
        object_pixels = find( Ilabel == k);
        object_area = size(object_pixels, 1); %same as total
        number of pixels in the object
        object_intensities = double(I(object_pixels)); %
15  need to convert to double to do math
        sorted_intensities = sort(object_intensities); %
        will need to get median, 20% and 80% pixels
        total_intensity = sum(object_intensities, 1);
        avg_intensity = total_intensity / object_area;
20      median_intensity = sorted_intensities( floor(
        object_area/2 ) + 1 );
        pix20 = sorted_intensities( floor(object_area*0.2)+1
        ) ; %brightest pixel among dimmest 20%
        pix80 = sorted_intensities( floor(object_area*0.8)+1
25      ) ;

        OData(k, 10) = total_intensity;
        OData(k, 11) = avg_intensity;
        OData(k, 12) = median_intensity;
30      OData(k, 13) = pix20; %brightest pixel among dimmest
        20%
        OData(k, 14) = pix80; %dimmest pixel among brightest
        20%
        end %for

```

```
%===== end function  
cmGetObjectsData() =====
```

5 (4). In a step 748, quantitative descriptors characterizing cell state are calculated based on the feature measurements extracted at step 746. For example, histogram distribution of intensities of cell nuclei provides information about the population cell cycle stages.

In a particular embodiment according to the present invention, data analysis techniques for describing the fluorescence patterns of cell portions in multiple cell lines in the presence and absence of compounds are provided. Automated image analysis techniques can include determining one or more regions from around nuclei, individual cells, organelles, and the like, called "objects" using a thresholding function. Objects that reside on the edge of an image can be included or excluded in various embodiments. An average population information about an object can be determined and recorded into a database, which can comprise a database text file or Excel spreadsheet, for example. However, embodiments can use any recording means without departing from the scope of the present invention. Values measured can be compared to the visual image. One or more types of numerical descriptors can be generated from the values. For example, descriptors such as a number of objects, an average, a standard deviation of objects, a histogram (number or percentage of objects per bin, average, standard deviation), and the like can be determined.

In a particular embodiment according to the present invention, data can be analyzed using morphometric values derived from any of a plurality of techniques commonly known in the art. For example, a software package called MetaMorph Imaging System, provided by Universal Imaging Corporation, a company with headquarters in West Chester, PA and NIH Image, provided by Scion Corporation, a company with headquarters in Frederick, Maryland.

30 Fluorescent images can be described by numerical values, such as for example, an area, a fluorescence intensity, a population count, a radial dispersion, a perimeter, a length, and the like. Further, other values can be derived from such measurements. For example, a shape factor can be derived according to a relationship

$4\pi \cdot \text{area} / \text{perimeter}$. Other values can be used in various embodiments according to the present invention. Such values can be analyzed as average values and frequency distributions from a population of individual cells.

In a particular embodiment according to the present invention, techniques for the automatic identification of mitotic cells are provided. Image analysis techniques employing techniques such as multidimensional representations, frequency-based representations, multidimensional cluster analysis techniques and the like can be included in various embodiments without departing from the scope of the present invention. Techniques for performing such analyses are known in the art and include those embodied in MatLab software, produced by MathWorks, a company with headquarters in Natick, MA.

Scalar values providing efficacious descriptors of cell images can be identified using the techniques of the present invention to perform predictive analysis of drug behavior. In a presently preferred embodiment, a plurality of heterogeneous scalar values can be combined to provide descriptors for each manipulation. By applying predictive analysis routines to the collections of these descriptors, predictive information about any number of manipulations and cell interactions can be extracted.

Fig. 7E illustrates a representative block flow diagram of simplified process steps for analyzing image feature values to obtain descriptors of cell state of step 718 of Fig. 7B in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. Fig. 7E illustrates an input data of descriptors of known manipulations 319. A step 320 of reformatting and transforming data 319 to formats suitable for analysis is performed. Additionally, a "cleaning" process can eliminate outlying data points and the like in the data. Then, in a step 322, a decision is made whether to continue with step 324 or with step 326 based upon determining a particular type of analysis appropriate for the present application or particular type of prediction. If decisional step 322 determines processing should continue with step 324, then, in that step, an error estimate using a set of test descriptors is performed to estimate the quality of a prediction and processing continues with step 320. Once an optimal prediction is achieved, processing continues with step 326. In step 326, optimal transformation parameters and prediction methods are selected for use in

steps 328 and 330 which analyze data about an unknown manipulation. In a step 328, a solution is generated based upon any of techniques including training a neural network, solving a mathematical equation, applying decision tree rules and/or the like. In a step 330, an input data set of unknown descriptors 318 is reformatted and
5 transformed based upon the optimal transformation parameters selected in step 326 using the transformation procedures in steps 320, 322 and 324. In a step 332, predictions techniques are applied to the reformatted manipulations from step 330 and the solution generated in step 328 and a plurality of properties of known manipulations 317 (e.g., therapeutic properties, and the like) in order to determine a
10 prediction of properties of unknown manipulation 316.

Fig. 7F illustrates a representative block flow diagram of simplified process steps for a method of mapping a manipulation of cells to a physiological characteristic in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein.
15 One of ordinary skill in the art would recognize other variations, modifications, and alternatives. The method is generally outlined by the steps below:

(1) In a step 750, a plurality of cells, e.g., dead, live, cell fractions or mixtures of cells are provided.

(2) Then, in a step 752, the plurality of cells is manipulated, where
20 manipulation occurs using a source(s) from one or a combination selected from an electromagnetic, electrical, chemical, thermal, gravitational, nuclear, temporal, or a biological source.

(3) Next, in a step 754, a feature value is captured from the plurality of cells. The feature value can include one or any combination of characteristics such as
25 cell count, area, perimeter, length, breadth, fiber length, fiber breadth, shape factor, elliptical form factor, inner radius, outer radius, mean radius, equivalent radius, equivalent sphere volume, equivalent prolate volume, equivalent oblate volume, equivalent sphere surface area, average intensity, total intensity, and optical density. This list is not meant to be limiting.

(4) Then, in a step 756, a degree of presence of one or more feature values is assigned for each manipulation.
30

(5) In a step 758, the feature values from the plurality of cells are stored in memory locations. From the memory locations the values can be used for

statistical analyses to produce predictive information about the relatedness of the descriptors of the manipulations to one another. This information is used to infer properties of the manipulations.

Fig. 7G illustrates a representative block flow diagram of a simplified process steps for a method for populating a database with manipulated biological cell information in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. The method is generally outlined by the steps below:

10 (1) In a step 760, a plurality of cells in various stages of the cell cycle, A montage image that was used as a source to generate data in Appendix A is presented in Fig. 12., such as for example, the stages of interphase, prophase, metaphase, anaphase, and telophase are provided.

(2) Then, in a step 762, each of the cells in the various stages of mitotic development is manipulated.

(3) Next, in a step 764, an image of the plurality of manipulated cells is captured using image acquisition techniques in order to provide a morphometric characteristic of each of the manipulated cells.

20 (4) As a preferable option, in a step 766, an image database may be populated with the image of the plurality of manipulated cells.

(5) Following step 764 or optional step 766, a morphological value is calculated from the image in a step 768.

(6) In a step 770, the database is populated with the morphological value.

25 Fig. 7H illustrates a representative block flow diagram of simplified process steps for a method for populating a database with manipulated biological information, e.g., image acquisition parameters, image feature summary information, and well experimental parameters in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. Fig. 7H illustrates a step 780 in which cells are placed into site on a plate and a manipulation is applied. Then, in a step 781 an image is taken of the cells. In step 782, the image is transferred to an image archive

30

database. Then, in a step 783, well experimental parameters are entered into the database 787. Well experimental parameters can include cell type, manipulation and the like. In a step 784, image acquisition parameters are transferred to database 787. Image acquisition parameters can include file name, fluorophores and the like. In a
5 step 785, the image acquired in step 781 is analyzed. Then, in step 786, an image feature summary from the analysis step 785 is transferred to database 787.

In step 788, a lookup table for all analyses is provided to database 787. The lookup table provides information about the analyses. In a step 789, a query of database 787 for process data is performed. The results are reformatted. Then in a
10 step 790, the database 787 is queried. Next, in a step 791, features of the manipulations stored in the database are combined and reduced. Next, in a step 793, reduced features of step 791 can be compared. In a step 792, the results of step 793 are recorded in database 787. Then, in a step 794, a report of predictions based on comparisons performed in step 793 is generated.

15 Fig. 7I illustrates a representative block flow diagram of simplified process steps for acquiring images of manipulated biological information, e.g., cells, cell tissues, and cell substituents in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations,
20 modifications, and alternatives. Fig. 7I illustrates a step 770 in which a user sets up an image analysis procedure. Then, in a step 772, an image is read into image analysis software. Next, in a step 774, patterns and objects are identified in the image using one or more algorithms. Next, in a step 776, sets of features are extracted from the image. Then, in a step 778, feature information, descriptor values and the like are
25 exported to the database, such as database 787 of Fig. 7H, for recording. Next, in a decisional step 779, a determination is made whether any more images should be taken. If this is so, processing continues with step 772. Otherwise, image acquisition processing is completed.

Fig. 7J illustrates a representative block flow diagram of simplified
30 process steps for populating, acquiring and analyzing images of manipulated biological information in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations,

modifications, and alternatives. Fig. 7J illustrates a step 300 of placing a plate onto an imaging stage and reading a bar code. Then, in a step 301 an autofocus procedure is performed. Next, in a step 302, a first optical filter configuration is selected and an image is collected. Then, in a decisional step 303, a determination is made whether
5 more than one image per optical configuration can be taken. If so, then, in a step 304, a new position within the well is targeted and another image is collected. Then, in a decisional step 305, a determination is made whether any more images need to be collected. If this is so, step 304 is repeated until all images for a particular well have been collected. After one or more images are collected for the well, in a step 306, the
10 stage is returned to a starting position within the well, and a montage is created from collected images. The results are named with a unique file name and stored.

In a decisional step 307, a determination is made whether any more optical channels in the well can be imaged. If this is so, then in a step 308 the next optical filter configuration is selected and an image is collected. Processing then
15 continues with decisional step 303, as described above. Otherwise, if no further optical channels in the well can be imaged, then in a decisional step 309 a determination is made whether any wells remain to be imaged. If not all wells have been imaged, then in a step 310, the stage moves to the next well and processing continues with step 301, as described above. Otherwise, if all wells on the plate have
20 been imaged, then in a decisional step 311, a determination is made whether any more plates can be processed. If this is so, then processing continues with step 300 as described above. Otherwise, in a step 312, the information is stored to a CD or other storage device as a backup.

Fig. 7K illustrates a representative block flow diagram of simplified
25 process steps compound based upon information about effects of one or more known compounds on a cell population in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. Fig. 7K illustrates a step 340 of populating a database
30 with descriptors for known compounds. Such descriptors can be determined from imaging the cell population. However, in some embodiments, descriptors can be derived by measurements and combinations of measurements and the like. Then, in a step 342, descriptors for the unknown compound are determined from imaging a

second cell population. The second cell population has been treated with the unknown compound. Then, in a step 344, a relationship between the descriptors determined from the unknown compound with the descriptors determined from the known compounds can be determined. Finally, in a step 346, an inference can be made about the unknown compound based upon the descriptors of the known compounds from the relationship determined in step 344.

Accordingly, the present invention provides a novel database design. In a particular embodiment according to the present invention, a method for providing a database comprises measurement of a potentially large number of features of one or more sub-cellular morphometric markers. Markers can be from any of a large variety of normal and transformed cell lines from sources such as for example, human beings, fungi, or other species. The markers can be chosen to cover many areas of cell biology, such as, for example markers comprising the cytoskeleton of a cell. The cytoskeleton is one of a plurality of components that determine a cell's architecture, or "cytoarchitecture". A cytoarchitecture comprises structures that can mediate most cellular processes, such as cell growth and division, for example. Because the cytoskeleton is a dynamic structure, it provides a constant indication of the processes occurring within the cell. The cytoarchitecture of a cell can be quantified to produce a one or more scalar values corresponding to many possible cellular markers, such as cytoskeleton, organelles, signaling molecules, adhesion molecules and the like. Such quantification can be performed in the presence and absence of drugs, peptides, proteins, anti-sense oligonucleotides, antibodies, genetic alterations and the like. Scalar values obtained from such quantification can provide information about the shape and metabolic state of the cell.

In a presently preferred embodiment, scalar values can comprise morphometric, frequency, multi-dimensional parameters and the like, extracted from one or more fluorescence images taken from a number of cellular markers from a population of cells. Two or more such scalar values extracted from a plurality of cell lines and markers grown in the same condition together comprise a unique "fingerprint" or descriptor that can be incorporated into a database. Such cellular descriptors will change in the presence of drugs, peptides, proteins, antisense oligonucleotides, antibodies or genetic alterations. Such changes can be sufficiently unique to permit a correlation to be drawn between similar descriptors. Such

correlations can predict similar properties or characteristics with regard to mechanism of action, toxicity, animal model effectiveness, clinical trial effectiveness, patient responses and the like. In a presently preferred embodiment, a database can be built from a plurality of such descriptors from different cell lines, cellular markers, and compounds having known mechanisms of action (or structure, or gene response, or toxicity).

The present invention also provides database and descriptor comparisons according to other embodiments. In a particular embodiment according to the present invention, measurement of scalar values or features can provide predictive information. A database can be provided having one or more "cellular fingerprints" comprised of descriptors of cell substance interactions of drugs having known mechanisms of action with cells. Such descriptors can be compared using a plurality of techniques, such as a technique of creating "phylogenetic trees" of a statistical similarity between the descriptors from various drugs. In a present embodiment, scalar, numeric values can be converted into a nucleotide or amino acid letter. Once converted into a corresponding nucleotide representation, the descriptors can be analyzed and compared using software and algorithms known in the art for genetic and peptide sequence comparisons, such as GCG, a product of Genetics Computer Group, with company headquarters in Madison WI. In an alternative embodiment, numeric values for the fingerprints can be used by comparison techniques. A phylogenetic tree can be created that illustrates a statistical significance of the similarity between descriptors for the drugs in the database. Because the drugs used to build the initial database are of known mechanism, it can be determined whether a particular scalar value in a descriptor is statistically predictive. Finally, a compound fingerprint with no known mechanism of action can be queried against the database and be statistically compared and classified among the drugs in the database that the compound most resembles.

In a particular embodiment, relationships between measured morphometric properties and features of images and physiological conditions can be determined. Relationships can include, for example, treatment of different cell lines with chemical compounds, or comparing cells from a patient with control cells, and the like. In a presently preferable embodiment, a clustering can be performed on acquired image descriptors. Some embodiments can comprise statistical and neural

network - based approaches to perform clustering and comparisons of various descriptors. The foregoing is provided as merely an example, and is not intended to limit the scope of the present invention. Other techniques can be included for different types of data. In some embodiments, clustering and comparing can be performed on features extracted from cell images. In a presently preferable embodiment, procedures for comparisons and phylogenetic analysis of biological sequences can be applied to data obtained from imaging cells.

Select embodiments comprising such approaches enable the use of a broad array of sophisticated algorithms to compare, analyze, and cluster gene and protein sequences. Many programs performing this task are known to those of ordinary skill in the art, such as for example, the program Phylip, available at <http://evolution.genetics.washington.edu/phylip.html>, and other packages listed at <http://evolution.genetics.washington.edu/phylip/software.html>. However, select embodiments according to the present invention can comprise a technique of statistical classification, statistical clustering, distance based clustering, linear and non-linear regression analysis, self-organizing networks, and rule-based classification.

Embodiments can perform such analysis based upon factors such as numerical value, statistical properties, relationships with other values, and the like. In a particular embodiment, numbers in a numerical descriptor can be substituted by one or more of nucleic acid or amino acid codes. Resulting "pseudo-sequences" can be subjected to analysis by a sequence comparison and clustering program.

Other types of databases can also be provided according to other embodiments. The database includes details about the properties of a plurality of standard drugs. When the descriptor of a test compound is compared to the database, predictions about the properties of the test compound can be made using any known property of the other compounds in the database. For example, properties about a compound in the database could include structure, mechanism of action, clinical side effects, toxicity, specificity, gene expression, affinity, pharmacokinetics, and the like. The descriptor of a compound of unknown structure from a natural products library could be compared to the descriptors of compounds with known structure and the structure could be deduced from such a comparison. Similarly, such information could lead to better approaches to drug discovery research including target validation

and compound analogizing, as well as pre-clinical animal modeling, clinical trial design, side effects, dose escalation, patient population and the like.

According to the present invention, databases can be integrated with and complementary to existing genomic databases. Differential genomic expression strategies can be used for drug discovery using database technology. In one particular embodiment, cell data and cellular response data can be associated with a genetic expression profile assay to form a single assay. Live cells expressing fluorescence markers can be treated with a drug, imaged and analyzed for morphometry; and then analyzed for mRNA for expression. Such embodiments can provide rapid development of tools to link cellular behavior with functional genomics.

Database methods according to the present invention can be used to predict gene function and to assist in target validation. Databases that include genetic diversity, i.e., having cellular descriptors from cells of differing genetic backgrounds (tumor, tissue specific, and gene knock out cell lines), can provide the capability to compare cells of unknown genetic background to those in the database. Similarly, the descriptor of an unknown cellular portion in the presence of multiple drugs can be queried against the descriptors of the known markers in the database. For example, if an unknown gene is tagged with Green Fluorescent Protein (GFP), the database may be used to identify the cellular portions for which that unknown gene encodes.

According to the present invention, target validation and specialized cell-based assay screening can be performed using database systems and methods to serve as a universal high-throughput cell-based assay that can evaluate the molecular mechanism of drug action. As new genes are isolated and identified, a large collection of available gene-based knowledge is becoming available. From this large collection of new genes, potential protein targets can be identified using the genomic tools of sequence analysis and expression profiling. However, unless a gene mutation is tightly linked to a disease state, further validation of individual targets is a time consuming process, becoming a bottleneck in drug discovery. Furthermore, robotics and miniaturization are making "High Throughput Screening (HTS)" the industry standard, substantially reducing the time and cost of running a target-based biochemical assay. Therefore, it is now possible to routinely screen large libraries and use a resulting "hit" to validate the target. In such approaches, a specialized cell-based assay would be developed to test hits for each target. Since this often involves

the creation of cell lines expressing new markers, this stage may also become a bottleneck that cannot keep pace with HTS. In addition, these cell-based assays may not be amenable to high-throughput screening, making it difficult to test the increasing number of analogs arising from combinatorial chemistry.

5 In a particular embodiment according to the invention, a rapid characterization of large compound libraries for potential use as pharmaceutical products can be provided by predicting properties of compounds that relate to the compounds' potential as bioactive drugs. In many drug discovery situations, virtually millions of compounds can be passed through a HTS assay against a small number of
10 validated targets. These assays produce hundreds to thousands of potential hits. These hits can then be subsequently screened by a pipeline of secondary and tertiary screens to further characterize their specificity, often time completely missing non-specific interactions with other proteins. Techniques according to the present invention can provide a replacement to such screening operations by providing
15 information about cellular accessibility and mechanism of action for the hits coming from a HTS system. Furthermore, it can replace the biochemical HTS assay and allow rapid and accurate identification of attractive compounds from large libraries without an intervening biochemical assay. The cell information can be predictive of whether to continue into an animal model for each compound, and which animal model to
20 pursue.

 The principles of the present specifically contemplate a wide variety of research methodologies, or usage scenarios, implementing these principles. The following discussion of three such scenarios is by way of illustration and not limitation. Study of the principles enumerated herein will render evident to those
25 skilled in the art certain additional methodologies or usage scenarios enabled by the teachings hereof. The present invention specifically contemplates all such modifications. The following description presents some specific embodiments and scenarios that represent a broader use of cellular phenotypic data and characterizations to deduce mechanisms of action and other features of cellular
30 responses to various stimuli. Such procedures generally involve producing a quantitative cellular phenotype based upon two or more cellular attributes and then comparing that phenotype to phenotypes previously stored and indexed. Such

procedures make use of databases or other repositories of biological information. The invention is not limited to the specific embodiments described here.

Considering first the procedure 2000 depicted in Figure 20, a compound has been identified as having a particular cellular activity. See 2004. For example, a compound may be found to inhibit the growth of certain cancer cell *in vitro* by a specific and desired mechanism of action. This may be a particular company's "gold standard."

Next, the compound is analyzed at 2006 in terms of its effect on one or more cell lines. More specifically, the compound is linked, virtually, to a particular phenotype. Two or more values or measures of cellular attributes characterize that phenotype. These attributes are quantified in the context of specific cellular markers.

In one example, the cellular marker is an organelle such as a nucleus or Golgi apparatus. Measured attributes useful for characterizing an associated phenotype include geometric parameters (e.g., size, shape, and/or location of the organelle) and composition (e.g., concentration of particular biomolecules within the organelle).

The phenotype may be characterized by administering the compound of interest to various cell lines and in various concentrations. In each example within this matrix, the attributes of interest are measured. Ultimately, certain phenotypic features (combinations of attribute values) are associated with the compound of interest. These features provide a template for the phenotype.

Next, using the phenotype as identified at 2006, the process identifies other compounds providing similar features. The goal here is to present a list of compounds having a mechanism of action similar to that of the compound that started the process. This allows researchers to identify a mechanism of action, if not already known, for their compound and to draw conclusions based upon their compound's link to other known compounds (which may not be chemically/structurally similar to the compound of interest).

Identifying similar compounds based upon phenotype can take many paths. Most will involve some mathematical basis. For example, the phenotype defined at 2006 can be represented as a fingerprint or vector comprised of multiple scalar values of cellular attributes (as described above). The phenotype representation can then be compared against known phenotypes characterized by the same format

(e.g., they are all characterized as vectors having the same attribute set, but with different values of the attributes). The comparison may be as simple as a Euclidean distance or more sophisticated as a neural network or multivariate statistical correlation.

5 The known compounds and associated phenotypes may be stored as database records or other data structures that can be queried or otherwise accessed as part of the identification procedure. The compounds may also be associated with other relevant data such as clinical toxicity, cellular toxicity, hypersensitivity, mechanism of action, etc. (when available).

10 Compounds found to be sufficiently similar to the starting compound are returned for consideration by researchers. A data processing system may rank such compounds based on degree of similarity to the starting compound. In some cases, the system may even provide similarity scores associated with the listed compounds.

15 Often researchers wish to determine whether their particular compound has clinical or biochemical effects beyond those that they are already aware of. In a typical scenario, the compound of interest was selected based upon its strong binding a target or its stimulation or inhibition of cell growth in a particular cell line. The process associated with 2010 has likely identified the compound of interest as having
20 a particular mechanism of action based on phenotypic similarity to other compounds having a similar mechanism of action. However, within the region of biochemical space, there may be subspaces (characterized by subphenotypes) that correspond to separate properties. For example, within the phenotypic space associated with one mechanism of action, there may be subspaces associated with clinical toxicity,
25 cellular toxicity (likely overlapping the clinical toxicity space), and little or no toxicity. Obviously, a researcher would like to know whether her compound is likely to be toxic.

 Thus, the process 2000 may include characterizing the compound of interest in terms of its distance from (i.e., similarity to) specific phenotypes having
30 known characteristics. In a typical example, the known characteristic is toxicity. This feature allows the researcher to quantify her compound in terms of mechanism of action AND toxicity (or in terms of two or more other relevant properties associated

with phenotype). To allow simple ranking or characterization, compounds of interest may be scored according to a simple or weighted Boolean expression.

A second scenario of interest is depicted in Figure 21. This scenario again defines a phenotype in terms of a quantifiable vector or other measure.

- 5 However, rather than using a compound of interest to generate the phenotype, some other cellular stimulus is used to generate the phenotype.

As shown, a process 2100 begins with receipt of cells of interest. See 2104. In many situations, the cells are produced by a genetic or epigenetic process that affects the expression level or activity of a particular protein. More generally,
10 any cellular stimulus (e.g., radiation level and type, gravity level, magnetic field, acoustic perturbations, etc.) can be used to generate the cell line of interest. Importantly, this stimulus affects the phenotype and can be correlated therewith.

In the context of drug discovery, a gene encoding for a particular target can be genetically knocked out, underexpressed, overexpressed, expressed in a non-
15 native state, etc. This may be accomplished via standard procedures involving genomic modification, translation or transcription apparatus modification (e.g., use of antisense nucleic acids), blocking target activity (using antibodies to a receptor site for example), and the like. These processes will generally affect the phenotype in some quantifiable way. Importantly, they clearly and unambiguously define a cellular
20 phenotype associated with altering the activity of the target protein.

At 2106, the process involves measuring one or more cellular features from the cell line of interest to define/quantify the phenotype. This may be accomplished as described above with reference to 2006. Next, at 2108, the cellular phenotype generated in this manner is used to identify and rank a set of compounds
25 associated with the phenotype. This operation may proceed in the manner of operations 2008 and/or 2010 from Figure 20.

Finally, at 2110, the process clusters the compounds returned at 2108 by a mechanism of action. The operation 2106 has tightly bound a mechanism of action to a phenotype. Various compounds characterized and stored in a system
30 database may be tentatively assigned a mechanism of action or may have no suggested mechanism of action. By matching their virtual phenotype to the phenotype generated at 2106, one can create or strengthen an association between the compounds and mechanism of action relevant to the stimulus at 2104.

Considering now Figure 22, a third scenario is depicted. This scenario again involves using a virtual phenotype to glean information relevant to a mechanism of action or other cellular activity. In this case, assay data from a group of compounds (e.g., a primary or focused library) is used to elucidate a phenotype.

5 As shown, a process 2200 begins by identifying a target protein. See 2204. Then, at 2206, the process involves identifying positive and negative biochemical hits. More generally, this may involve ranking a number of compounds based upon their interaction with the target. In a specific case, the compounds are ranked based upon their binding affinities to or ability to inhibit the enzymatic activity
10 of the target protein.

After the compounds have been characterized in some manner based upon their interaction with the target, they are used to define a cellular phenotype. See 2208. Generally, the techniques to accomplish are the same as described with reference to operation 2006 of Figure 20. In this case however, one may obtain a
15 strong correlation between mechanism of action (involving the target) and phenotype by using multiple of the compounds identified at 2206. For example, some of the "best hits" may be administered to cell lines in various concentrations. And some of the least effective compounds may also be administered. Cellular attributes that are more strongly exhibited with increasing concentration of the best hits (and not
20 exhibited or exhibited only weakly upon administration of the negative hits) can be used to define the virtual phenotype. In a related approach, compounds having widely varying levels interaction with the target are administered to cells. Those cellular attributes that vary linearly or at least monotonically with the degree of interaction between the target and compound represent attributes that can be used to define the
25 virtual phenotype.

After the cellular phenotype has been defined, previously characterized compounds may be clustered with that phenotype. See 2210. As with operation 2110 of Figure 2, this may create or strengthen an association between a mechanism of action and various compounds in a database.

30 Finally, and optionally, procedure 2200 may provide a "higher resolution" mechanism of action for the compounds identified at 2206. See 2212. Presumably interaction with the target suggests a specific mechanism of action or at least some aspect of a mechanism of action. However, a given target may participate

in a larger cellular mechanism of action – unknown to researchers. Further, a compound may that binds with the target may participate in multiple mechanisms of action – some of which do not involve the target. By linking the target (and its positive hits) to a particular phenotype, some of these additional cellular level activities can be elucidated. The defined phenotype may have been previously identified as associated with other mechanisms of action or higher resolution mechanisms of action. Thus, the phenotype identified at 2208 can be leveraged to generate a higher resolution mechanism of action at 2212.

As suggested in the above discussion, compounds and associated phenotypes may be stored as database records. Such databases can take on many flavors. In one example, a database includes various pieces of information relevant to oncology. Such database may include numerous compounds classified by cellular phenotype, mechanism of action, toxicity, etc. More specifically, the database may include data on commercially available compounds clustered by cellular phenotypes corresponding to mechanisms of action. Further the databases of interest may extended or combined (via standard relational tables and algebra for example) to include additional data such as pharmacology data, cellular genomics data, gene expression data, protein expression data, etc. In a specific example, the database includes measurements made on a subset of the NCI60 cell lines, using DNA, Golgi apparatus, and/or microtubules as markers for defining the phenotypes. Other data includes dosage response information, variation in effect over time, etc. The compounds populating the database could include known National Cancer Institute oncology study compounds. In a specific embodiment, the compound set includes some or all of the compounds mentioned in the article “A gene expression database for the molecular pharmacology of cancer,” Nature Genetics, 24, pp. 236-244 (March 2000).

Various biological analyses may be conducted to develop additional information for characterizing compound mechanisms of action, etc. For example, a cell count analysis may be used to develop dose response curves, GI 50 data, etc. The cell cycle may also be analyzed to find out how various stages in the cycle vary in response to particular stimuli. The Golgi apparatus may be analyzed to determine whether it is in a normal state, a dispersed state, a diffused state, etc. As another example, tubulin may be analyzed to determine whether it is normal, de-polymerized,

over-polymerized, bundled, etc. Obviously, combinations of such analyses may be performed. For example, properties of the Golgi apparatus or tubulin may be analyzed over one or more cell cycles.

In some embodiments, techniques according to the present invention
5 can provide tools for the later stages of drug development such as clinical trial design and patient management. The properties of known drugs, such as clinical trial and patient response information, will be used in a similar fashion as the pre-clinical information to provide predictions about the properties of novel compounds. Because the human cell is the locus of drug action, a database containing drug-cell interactions
10 will be able to provide predictive value for this aspect of drug development.

Although the above has generally been described in terms of specific hardware, software, and methods, it is understood that many alternatives can exist. In particular, the present invention is not limited to a particular kind of data about a cell, but can be applied to virtually any cellular data where an understanding about the
15 workings of the cell is desired. Thus, in some embodiments, the techniques of the present invention could provide information about many different types or groups of cells, substances, and genetic processes of all kinds. Of course, one of ordinary skill in the art would recognize other variations, modifications, and alternatives. Some examples according to the present invention are provided below.

20

EXPERIMENTS

To prove the principle and demonstrate the objects of the present invention, experiments have been performed to determine the effects of manipulations on cell structure using imaging and analysis techniques applied to a variety of
25 situations. These experiments were performed by growing multiple cell lines in the presence of multiple compounds, or substances. Cells were fixed and stained with fluorescent antibodies or labels to multiple cellular portions. One or more images of the cells were then obtained using a digital camera. Descriptors were built by quantifying and/or qualifying patterns of one or more feature from each image in the
30 cell lines under study. A database was built from the descriptors. As the database grows, it should be able to predict the mechanism of action of an unknown drug by comparing its effect with the effects of known compounds or to identify data clusters within large libraries of compounds.

In a first experiment, an automated method to count the number of cells and differentiate normal, mitotic, and apoptotic cells was created.

Approximately, 5,000 HeLa cells were plated per well in a 96 well plate and grown for 3.5 days. The cells were fixed with -20° MEOH for 5 minutes, washed with TBS for 15 minutes, and then incubated in 5 mg/ml Hoechst 33342 in TBS for 15 minutes. Then, 72 images were collected with a 40x objective and 75 ms exposure time.

The analysis was performed on objects that met a certain size criteria that was based on 1) measuring the size of objects in the image that were clearly not cells and 2) excluding the first peak of the area histogram (Fig. 8B values 1-4654).

Histograms of the individual object data were generated for each type of feature. Fig. 8A shows the histogram for average intensity, and Fig. 8B shows histogram data for the area of each object. Fig. 8C shows the scatter plot of the average intensity vs. the area of all of the objects. The pattern of the scatter plot showed an interesting pattern: a large cluster of cells in one region of the graph, with a scattering of object points in other regions. Because mitotic structures are identified as particularly bright objects, most likely due to the biological fact that the chromatin is condensed, the original Hoechst images could be used to identify which cells were either undergoing mitosis, or otherwise looked abnormal. Manual inspection of 917 cells resulted in the classification of each object. Fig. 8D shows a graph where each type of cellular classification is delimited. This graph clearly shows that the mitotic nuclei are brighter than the interphase nuclei. Further, the different phases of the cell cycle can be separated using these two features. Figs. 8E-8F show bar graphs of the average and standard deviations of the areas and average intensities for each cell classification type. These graphs show that interphase nuclei are statistically less bright than mitotic nuclei and that telophase nuclei are statistically smaller than other mitotic nuclei.

Each image was thresholded to an intensity level of 20. A standard area value was set at 9500 pixels. Automated information gathering about all of the objects was done and collected into an Excel spreadsheet (for more information see, section on imaging system). The following information was recorded:

IMAGE NAME
OBJECT #

AREA
STANDARD AREA COUNT
PERIMETER
FIBER LENGTH
FIBER BREADTH
SHAPE FACTOR
ELL. FORM FACTOR
INNER RADIUS
OUTER RADIUS
MEAN RADIUS
AVERAGE INTENSITY
TOTAL INTENSITY
OPTICAL DENSITY
RADIAL DISPERSION
TEXTURE DIFFERENCE MOMENT
EFA HARMONIC 2, SEMI-MAJOR AXIS
EFA HARMONIC 2, SEMI-MINOR AXIS
EFA HARMONIC 2, SEMI-MAJOR AXIS
ANGLE
EFA HARMONIC 2, ELLIPSE AREA
EFA HARMONIC 2, AXIAL RATIO
EFA HARMONIC 3, SEMI-MINOR AXIS

The following results were obtained:

- 1,250 objects were counted
- 201 of those objects has standard area counts > 2 (area > 19000 pixels)
- 195 objects had areas < 6000 pixels
- 1529 objects estimated in total
- 1328 object areas are > 6000 pixels
- The data was reduced to 917 objects that were $6000 < \text{area} < 19000$
- For the 917 objects a scatter plot of area vs. average intensity and a histogram of the average intensity were generated.

- 116 objects that had average intensity intensities > 60 were manually looked at to determine their morphology.
 - Of those 116 objects:
 - 6 were dead or indistinguishable
 - 4 were interphase
 - 30 were prophase
 - 32 were metaphase
 - 24 were anaphase
 - 20 were telophase (10 pairs)
- 10
- 12 prophase objects were missed because of gray scale cut off. (8 of those prophase cells had gray scale values > 57 , as did 7 interphase)
 - 1 telophase object was missed because it was too small (< 6000)
 - 1 prophase object was missed because it was too big (> 1900)
- 15
- 16 mitotic objects were missed because they were parts of objects with standard count > 2 .

In sum, out of 917 single objects, the analysis correctly identified 106 out of 130 mitotic objects, or (81% predictive, 91% of identified mitotics). Out of 917 single objects, the analysis incorrectly identified only 10 non-mitotics as mitotics (1% total, 8% of identified mitotics); 14 mitotics as interphase (1.4% total, 1% interphase). An automated classification system that would automatically assign values to each object using these or other measurement features can thus be developed, utilizing the principles set forth herein.

In a second experiment, the effects of Taxol on MDCK cells and the different types of morphological effects were observed. A plurality of MDCK cells grown in 96 well plates were treated with Taxol for 4.5 hours at different concentrations (10 uM-1pM). They were then fixed, labeled with Hoechst, and imaged.

This experiment used a labeling protocol comprising: MEOH fix at – 20°, Wash in PBS, Block in PBS/BSA/Serum/Triton-X 100, Incubate with 5 µg/ml Hoechst 10 minutes, and wash.

Cells were inspected for different morphologies and manually counted at each different drug concentration in one well. Fig. 9 shows example images from each drug concentration and the different types of morphologies and cells are highlighted. Fig. 10 shows the distribution of each morphology within the cell population as a function of drug concentration. The higher the concentration of Taxol, the larger proportion of cells underwent apoptosis, and the fewer number of normal mitotic cells were detected.

In a third experiment, the purpose was to determine whether the automated analysis methods developed in the first experiment can detect differences in Hoechst morphology in the presence of 6 known compounds at one concentration and exposure time in one cell line. In this experiment, HeLa cells were separately treated with 6 compounds with known mechanism of action. The quantitative methods described in the first experiment were applied to the Hoechst images.

Approximately 5,000 HeLa cells per well were plated in a Costar black-walled 96 well tissue culture treated plate and left to recover in the incubator for 24 hours. After this time, 10 ug/mL of cytochalasin D (CD), Taxol, hydroxyurea, vinblastine, nocodazole, and staurosporine was added to different wells at a 1:100 addition in DMSO.

The cells were incubated in the presence of drug for 24 more hours. After 24 hours, the cells were removed and fixed as in the first experiment. Then, 9 images per well were collected of the Hoechst staining using a 10x objective.

The low magnification images taken of Hoechst were run through the automated image analysis method described in the first experiment. Plots of the average intensity and area were made of each compound. Fig. 11 shows the scatter plots of the compounds. The scatter plots of each compound are visually distinct. For example, cells treated with CD are smaller than control, and cells treated with Hydroxyurea are larger and brighter. Furthermore, the number of cells per well was very different (data not shown).

The effects of different compounds can be clearly and automatically distinguished by identifying changes in cellular morphology. This method can also be used to count adherent cells.

The next experiment was to develop clustering algorithms that assign statistically meaningful values to the representative two dimensional data shown in Fig. 10, and even more complicated clustering of all of the multidimensional data that can be extracted across one, and multiple images.

A fourth experiment was performed to obtain high magnification images of two markers in the presence of drugs. In this experiment, HeLa cells were treated with 80 generic compounds with known mechanism of action. The quantitative methods described in the first experiment were applied to the Hoechst images.

Approximately 5,000 HeLa cells per well were plated in a Costar black walled 96 well tissue culture-treated plate and left to recover in the incubator for 24 hours. After this time, 10 ug/mL of each compound from the Killer Plate from Microsource Discovery Systems (Gaylordsville, CT) was added to different wells at a 1:100 addition in DMSO. The cells were incubated in the presence of drug for 24 more hours. After 24 hours, the cells were removed and fixed as in the first experiment. In addition to being labeled with Hoechst 33342 (against chromatin), cells were also labeled with 1 unit of rhodamine-conjugated phalloidin (against actin) for 30 minutes.

The 96 well plate was imaged twice. Once, 9 images per well were collected of the Hoechst staining using a 10x objective. After this, one image per well of both the phalloidin and Hoechst staining was collected using a 40x objective.

The resulting high magnification images were analyzed qualitatively and distinct pattern differences were detected in both the Hoechst and phalloidin images. Fig. 12 shows three example images from the experiment. The top row is the Hoechst staining, and the bottom row is the phalloidin staining from the same well. The columns show the images from wells treated with just DMSO (control), cytochalasin D, and Colchicine. The morphology of each marker is different in the presence of each drug. Interestingly, there is an effect in the morphology of the chromatin in the Hoechst image of cytochalasin D, which directly targets the actin cytoskeleton (and thus there is an expected effect in the phalloidin image). Also, there is an effect on the actin cytoskeleton, compared to control, in the presence of colchicine that directly targets the microtubule network.

The low magnification images were analyzed as described in the first experiment, and different patterns were seen in both the average intensity vs. area plots, and in the number of cells per well (data not shown). Thus, changes in patterns of a marker that is "down-stream" from the direct target of a compound are detectable. Automated image analysis protocols for actin and other markers can be developed similarly, again utilizing the principles set forth herein.

A fifth experiment was performed to test quadruple labeling of 9 different cell lines grown in normal conditions. In this experiment, NCI-H460, A549, MDA-MD-231, MCF-7, SK-OV-3, OVCAR-3, A498, U-2 OS, and HeLa cells were plated. Then, the cells were fixed and stained for portions of the each cell known as DNA, tubulin, actin, and Golgi.

The following table summarizes the procedures for this experiment:

Action	Active Ingredient/Notes	Buffer	Vol/ well	Desired Time	Temp
Remove media	NOTE: gently by pipetting, not aspiration				
Fix	4% Formaldehyde	PBS	100µl	20 min	rt
Wash		TBS	100µl	5 min	rt
Wash		TBS	100µl	5 min	rt

Permeablize	0.1% Triton X-100	TBS	100μl	10 min	rt
Permeablize	0.1% Triton X-100	TBS	100μl	10 min	rt
Block	% BSA % Serum Filter sterilize before use	TBS w/azide	100μl	1hr or o/n	rt or 4°C
Primary Antibody	1:1000 dilution of DM1α	TBS + 1% BSA + 0.1% TX-100	50μl	1hr or o/n	rt or 4°C
Wash		TBS	100μl	5 min	rt
Wash		TBS	100μl	5 min	rt
Wash		TBS	100μl	5 min	rt
Fluorescent Stain	FITC lens culinaris 1:500 Rhodamine-Phalloidin 1:500 CY5 goat anti-mouse 1:100	TBS + 1% BSA + 0.1% TX-100	50μl	1 hr.	rt, dark
Wash		PBS	100μl	5 min	rt, dark
Hoechst	1:1000 dilution of 5mg/ml	TBS	100μl	15 min	rt, dark
Wash		PBS	100μl	5 min	rt, dark
Wash		PBS	100μl	5 min	rt, dark
Wash		PBS	100μl	5 min	rt, dark
Store		PBS	200μl	1 month	4°C

Cells were plated out at different densities for 48 hours. Cells were fixed and labeled by the above method. Cells were imaged using an automated imaging system that collected 9 images from each marker using a 10x objective.

Higher magnification images were collected of a few cells for demonstration purposes.

In this experiment, each cell line demonstrated different morphological patterns as determined by phase. For example, A549 cells are much more compacted than OVCAR-3 cells as determined by phase contrast imaging (data not shown). The different fluorescent markers showed even bigger differences between different cell lines. Figs. 13 and 14 show 4 panels of each marker for A549 (Fig. 13) and OVCAR-3 cells (Fig. 14). The markers are Hoechst (upper left), Phalloidin (upper right), Lens culinaris (lower left), and DM1a antibody (lower right). The following table summarizes the qualitative differences between these images:

MARKER	A549	OVCAR3
Hoechst/DNA	small	large
Phalloidin/actin	fuzzy	crisp - many stress fibers
Lens culinaris/Golgi	compact	Disperse/punctate
DM1alpha/Tubulin	perinuclear	evenly distributed

Higher magnification images were taken of the OVCAR3 cells. Fig. 15 shows the same markers at 20x, and Fig. 16 shows the markers at 40x. While the highest magnification images show the most detail, these images illustrate that very little morphological or feature information is lost in the 10x images.

These data exemplify the differences in morphology seen between different cell types. Thus the automated image analysis software can be customized for each marker in each cell type. Different drugs should effect these morphologies differentially.

An automated quantification method for each marker and cell line can be similarly developed.

A sixth experiment was conducted with a more sophisticated software package and to develop more flexible image recognition algorithms. In this experiment, prototype image features extraction was performed using MatLab programming language with image toolbox and SDC morphology toolboxes. Algorithms are being developed that will automatically identify objects on images and

to measure various morphological and feature parameters of these objects. Many different features for each of the cellular markers were acquired.

An example of a MatLab program called "AnalyseDNA" that takes as an input an unlimited number of images, identifies individual objects in these images based on either their intensities, or based on edge-detection algorithms, and extracts a number of morphological and intensity characteristics of these objects. A copy of this program follows:

Listing of the AnalyseDNA.m program and of some of the
supporting subroutines

```

10 function files_analysed = AnalyseDNA(filemask, outpath,
    nx, ny, filter_range, dext, modifier, sfname)
    % AnalyseDNA performs measurements on files of DNA images
    % V1. EV 2-11-99; 2-15-99; 2-16-99
15 %
    % files_analysed = AnalyseDNA(filemask, outpath, nx, ny,
    filter_range, dext, modifier, sfname)
    %
    % PARAMETERS:
20 %     ALL PARAMETERS ARE OPTIONAL
    %
    %     FILEMASK - mask for file names to be analyzed
    INCLUDING PATH(for example c:\images\*.tif)
    %     DEFAULT '*.tif' (all *.tif files in the current
25 directory).
    %
    %     OUTPATH - path to a directory where all the output
    files will be placed.
    %     DEFAULT - output is saved in the same directory
30 which contains images
    %
    %     NX, NY - number of individual images in montage
    images along X and Y axes (DEFAULT 1)
    %

```

```
%    FILTER_RANGE - 3 col-wide array (or[]). Specifies
%    how data is filtered when summary is calculated
%    this parameter internally is passed to GetDNADData
%    and then to GetSummaryData - see these
5 %    functions for details. For example: [2 2 Inf; 6 100
%    8000] will case all rows of data for which
%    values in column 2 are less than 2 and all rows
%    where values in column 6 are less than 100 or
%    more than 8000 to be excluded from all
10 calculations of a summary.
%    DEFAULT - [] (means do not filter, summarize all
%    data)
%
%    DEXT - string. Extension for data files being saved.
15 %    DEFAULT 'dat';
%
%    MODIFIER - this modifier is 'SUMMARY', summary file
%    is created;
%    'SUMMARY ONLY' - only summary is generated,
20 data for individual files are not saved
%
%    sfname - string. File name of a summary file
%    DEFAULT 'summary[date].dat'
%
25 % OUTPUT:
%
%    AnalyseDNA works on image files or montages. For
%    each image file it creates a tab-delimits file of
%    measured
30 %    parameters of all the objects in the montage with
%    the same base name as a montage file and extension
%    specified
```

```
%      by dext parameter (or .dat by default) and file
'errors[date].err' - with the list of files that matched
the
%      filemask but could not be processed.
5 %      If 'summary' or 'summary only' modifier is
specified, it also creates a single file
'summary[date].dat' (or
%      different extension, if specified by DEXT) which
contains summary information for all analyzed files.
10 %
%      ALL OUTPUT FILES are saved in a directory specified
by OUTPATH parameter
%
%      RETURNS *files_analysed* - number of files that have
15 been successfully processed.
%
%      Column designations in the output files are
described in GetDNADData
%
20 % FILE NAME CONVENTIONS
%      AnalysedDNA attempts to identify a number for each
file to identify the file in summary output.
%      It does that by looking for the first space or
underscore, followed by a number and then takes
25 %      as many successive numbers as it can find. If it
fails to identify a number it assigns a
%      default which is -1
%
%
30 % SEE ALSO GetDNADData, GetSummaryData
%
% TO DO      improve error handling in opening and writing
files (GLOBAL error_file ?)
```

```
%      include procedures for writing text headers
into the output files

if nargin > 8
5      error ('Wrong number of input parameters');
end
if nargin > 1
    error ('Wrong number of output parameters: only one
allowed');
10 end

% set defaults
need_summary = 0;
summary_only = 0;
15 use_default_outpath = 0;
datestring = datestr(floor(now));
if nargin == 7      % set default summary file name
    sfname = ['summary' deblank(datestring)]; % extension
will be appended later based on dext
20     if deblank(upper(modifier)) == 'SUMMARY'
        need_summary = 1;
        elseif deblank(upper(modifier)) == 'SUMMARY ONLY'
            need_summary = 1;
            summary_only = 1;
25     else
        error (['Wrong parameter: unknown modifier '
modifier]);
        end
    end
end
30
if nargin == 5
    % default data file extension
    set_dext = 'dat';
end
```

```
    if nargin == 4
        % default filter range
        filter_range = [];
    end
5   if nargin == 3
        ny = 1; % default number of images in montage along Y
    end
    if nargin == 2
        nx = 1;
10   end
    if nargin == 1
        use_default_outpath = 1;
    end
    if nargin == 0
15     filemask = '*.tif'
    end

    % check parameters
    if ( ~ischar(filemask) | ~ischar(dext) | ~ischar(sfname)
20   )
        error('Wrong parameter type: filename, filepath,
dext and sfname should be strings');
    end
    if ( ( size(nx) ~= [1 1] ) | ( size(ny) ~= [1 1] ) )
25     error ('Wrong parameter type: nx and ny should be
scalars (1x1 arrays)');
    end
    if (~isempty(filter_range) & size(filter_range, 2) ~= 3)
        error ('Wrong parameter type: filter range should be
30  [] or 3 - cols-wide array');
    end
    % end testing parameters

    % Generate list of files to process
```

```
datapath = getpath(filemask);
if use_default_outpath == 1
    outpath = datapath;
5  end
if exist(outpath, 'dir') ~= 7
    error(['Path ' outpath, 'not found. Exiting..']);
elseif exist(datapath, 'dir') ~= 7
    error(['Path ' datapath, 'not found. Exiting..']);
10 end

sfname = makefullname(outpath, sfname, dext);
if need_summary == 1
    if exist(sfname, 'file')
15         disp(['File ', sfname, 'already exists!']);
        input ('Press ^C to abort, Enter to delete and
continue');
        delete(sfname);
    end
20 end

flist = FileList(getfname(filemask), datapath);
numfiles = size(flist, 1); % total number of files to
25 process
disp(['About to process ', num2str(numfiles), ' files']);
%DEBUG - commented out "input" to run from Wrod
input('Press ^C to abort, Enter to continue');

30 % main loop where the job gets done:
error_file = makefullname(outpath, ['error' datestring
'.err']);
num_processed = 0;
num_error = 0;
```

```
for i = 1:numfiles
    % first generate file name for a data output file
    current_fullname = flist(i, :); % full name with path
    and extension
5    current_datafile = makefullname(outpath,
    makefname(getbasefname(current_fullname), dext) );

    %extract number from a filename
    fnumber = getfilenumber(current_fullname);
10

    % load an imagefile, record errors
    read_error = 0;
    try
        I = imread(current_fullname);
15        %DEBUG
        disp (['Image file #', num2str(fnumber), '
loaded']);
        catch
            % record file-opening error in an error_file
20            read_error = 1;
            num_error = num_error +1;
            msg = [current_fullname ': ' lasterr];
            add_error_msg(error_file, msg);
        end

25
        % extract and write data to a file in outpath
        if read_error ~=1
            if (need_summary == 0)
                %DEBUG
30                disp (['Starting analysis of file #',
num2str(fnumber), '.']);
                current_data = GetDNADData(I, nx, ny, fnumber);
                %DEBUG
```

```

        disp (['Finished analysis of file #',
num2str(fnumber), '.']);
        %load current_data.mat 'current_data';
        write_data(current_data, current_datafile);
5         else      %summary needed
            %DEBUG
            [current_data, current_summary] = GetDNADData(I,
nx, ny, fnumber, filter_range);
            %load current_data.mat 'current_data';
10         %load current_summary.mat 'current_summary';
            write_summary (current_summary, sfname);
            if summary_only ~= 1
                write_data(current_data, current_datafile);
            end
15         end
        end
    end % of the main for loop
    num_processed = numfiles - num_error;

20  %=====end function AnalyseDNA()
    %=====

    %=====
    %=====

25  function result = add_error_msg(filename, msg)
    % adds string MSG to an errorfile FILENAME
    % returns 1 if success, 0 if failure

    err_FID = fopen(filename, 'at');
30  if err_FID == -1
        warning(['Can not open error file ' filename]);
    else
        fprintf(err_FID, '%s\n', msg);
        fclose(err_FID);

```



```

end

%=====end function add_error_masg()
=====

5  %=====
=====

function N = getfilenumber(fname)
% returns the first number extracted from a file name
% (string) or -1 if fails to extract any number
10 numbers = NumbersFromString( getfname(fname) ); % vector
    of all numbers encoded in the name

                                % (but not in the path, even if
                                present)
15 if isempty(numbers)
    N = (-1);    % return -1 if no numbers found in the
    name
    else
        N = numbers(1);
20 end

%===== end function getfilenumber()
=====

25 %=====
=====

function result = write_data(data_array, file_name)
% writes data in a data_array in a tab-delimited ascii
file.
30 % result is 0 if success and -1 if failure
% if file_name exists, overwrites it
result = -1;
try
    fid = fopen(file_name, 'wt');

```

```

        if fid ~= -1
            for k = 1:size(data_array, 1)
                fprintf(fid, '%g\t', data_array(k, :));
                fprintf (fid, '\n');
5         end
        test = fclose(fid);
        result = -1;
    catch
        result = -1;
10    end

    %===== end function write_data()
    %=====

15    %=====
    %=====
    function result = write_summary (s_vector, file_name)
    % appends summary vector s_vector to a file_name (ASCII
    tab-delimited file).
20    % if file_name does not exist, creates it.
    % result is 0 if success and -1 if failure
    %
    result = -1;
    try
25        % debug
        fid = fopen(file_name, 'at');
        result = fprintf(fid, '%g\t', s_vector);
        result = fprintf(fid, '\n');
        result = fclose(fid);
30        result = 0;
    catch
        result = -1;
    end

```

```

% ===== end function write_summary()
=====

function Data = GetObjectsData(I, Ilabel)
5 % GetObjectsData returns array measurements of objects in
  image "I" masked by "Ilabel"
  % EV 2-3-99; 2-10-99
  % OData = GetObjectsData(I, Ilabel) returns an array of
  morphological and intensity measurements
10 %   taken from a grayscale image "I". Objects are
  identified on a mask image Ilabel, usually
  %   created by bwlabel()
  % OUTPUT:
  % Each row in the output array OData represents
15 individual object
  % columns contain the following measurements:
  %
  %   1 - Index ("number" of an object);      8 -
  Solidity;
20 %   2 - X coordinate of the center of mass; 9 - Extent;
  %   3 - Y coordinate      "-"; 10 - Total
  Intensity;
  %   4 - Total Area (in pixels);      11 - Avg.
  Intensity;
25 %   5 - Ratio of MajorAxis/MinorAxis;      12 - Median
  Intensity;
  %   6 - Eccentricity;      13 - Intensity of
  20% bright pixel
  %   7 - EquivDiameter;      14 - Intensity of
30 80% bright pixel
  %
  % For details on morphological parameters see information
  on MatLab imfeature();

```

```
% Intensity parameters are either obvious or are
documented in comments in this file.

% Procedures in this file are documented in notebook file
"MATLAB Measuring Nuclei (1) 1-29-98.doc"

5
if (nargin ~= 2)
    error ('function requires exactly 2 parameters');
end
if (nargout ~= 1)
10    error ('function has 1 output argument (array X by
    14)');
end

% finished checking arguments

15
% first collect morphological parameters in a structure
array:
ImStats = imfeature(Ilabel, 'Area', 'Centroid',
    'MajorAxisLength',...
20    'MinorAxisLength', 'Eccentricity', 'EquivDiameter',
    ...
    'Solidity', 'Extent', 8 );

% now convert it into array (matrix) while collecting
25 intensity data for each object:

%preallocate output array:
numobjects = size(ImStats, 1);
OData = zeros(numobjects, 14);
30 %now convert ImStats into array and add intensity data to
it
for k=1:numobjects
    OData(k, 1) = k;
    OData(k, 2) = ImStats(k).Centroid(1);
```

```

        OData(k, 3) = ImStats(k).Centroid(2);
        OData(k, 4) = ImStats(k).Area;
        OData(k, 5) = (ImStats(k).MajorAxisLength) /
        (ImStats(k).MinorAxisLength);
5         OData(k, 6) = ImStats(k).Eccentricity ;
        OData(k, 7) = ImStats(k).EquivDiameter;
        OData(k, 8) = ImStats(k).Solidity;
        OData(k, 9) = ImStats(k).Extent;

10         % now collect and assign intensity parameters from
        image I

        object_pixels = find( Ilabel == k);
        object_area = size(object_pixels, 1); %same as total
15 number of pixels in the object
        object_intensities = double(I(object_pixels)); %
        need to convert to double to do math
        sorted_intensities = sort(object_intensities); %
        will need to get median, 20% and 80% pixels
20         total_intensity = sum(object_intensities, 1);
        avg_intensity = total_intensity / object_area;
        median_intensity = sorted_intensities( floor(
        object_area/2 ) + 1 );
        pix20 = sorted_intensities( floor(object_area*0.2)+1
25 ) ; %brightest pixel among dimmest 20%
        pix80 = sorted_intensities( floor(object_area*0.8)+1
        ) ;

        OData(k, 10) = total_intensity;
30         OData(k, 11) = avg_intensity;
        OData(k, 12) = median_intensity;
        OData(k, 13) = pix20; %brightest pixel among dimmest
        20%

```

```

        OData(k, 14) = pix80; %dimmet pixel among brightest
    20%
    end %for

5   %===== end function
    GetObjectsData()=====

function Imask = MaskDNA1(I);
10  % MaskDNA1 - generates binary mask for cell nuclei
    through edge detection
    % EV 1-22-99; 2-6-99; 2-10-99
    % Imask = MaskDNA1(I)
    % PARAMETERS
15  %   I - intensity image (grayscale)
    % OUTPUT
    %   Imask - BW image with objects from I
    %
    % For more details see Notebook Matlab_DNA_masking1_1-22-
20  99.doc
    % Uses SDC Morphology Toolbox V0.7

    if (nargin ~= 1)
        error('Wrong number of input parameters');
25  end
    if (nargout ~= 1)
        error('Wrong number of output parameters: one output
        argument should be provided');
    end
30

    Imask = edge(I, 'canny');
    Imask = mm dil(Imask, mmsecross(1));
    Imask = mmerc ( mmc lohole(Imask,mmsecross(1)));

```

```

Imask = mmedgeoff(Imask, mmsecross(1));
% note that mmedgeoff this command removed FILLED OBJECTS
but not touching OUTLINES.
% these outlines can be removed by filtering:
5  Imask = medfilt2(Imask, [5 5]);

%=====end MaskDNA1 =====

```

Given the list of image files or montages of images as an input, this
 10 program creates an individual file for each image that contains the following
 quantitative measurements for all objects identified in the image:

- | | |
|---|------------------------------------|
| 1 - Index ("number" of an object); | 8 - Solidity; |
| 2 - X coordinate of the center of mass; | 9 - Extent; |
| 15 3 - Y coordinate "-"; | 10 - Total Intensity; |
| 4 - Total Area (in pixels); | 11 - Avg. Intensity; |
| 5 - Ratio of MajorAxis/MinorAxis; | 12 - Median Intensity; |
| 6 - Eccentricity; | 13 - Intensity of 20% bright pixel |
| 7 - EquivDiameter; | 14 - Intensity of 80% bright pixel |

20 A fragment of an output for a single file, containing 9 images of cells
 stained for DNA and acquired with a 10x objective. A montage image that was used
 as a source to generate data in A is presented in Fig. 17.

The same program also summarizes measurements across many files
 and performs statistical analysis of the summary data. It creates a summary file with
 25 the following data:

- | | |
|--|---------------------------------|
| 1 - Image file number; | |
| 2 - Average object Area (in pixels); | 3 - STD (standard deviation) of |
| 2; | |
| 30 4 - Avg. of Ratio of MajorAxis/MinorAxis; | 5 - STD of 4; |
| 6 - Avg. Eccentricity; | 7 - STD of 6; |
| 8 - Avg. EquivDiameter; | 9 - STD of 8; |
| 10 - Avg. of Solidity; | 11 - STD of 10; |

- | | |
|---|----------------|
| 12 - Avg. of Extent; | 13 - STD of 11 |
| 14 - Avg. of objects Total Intensity; | 15 - STD of 14 |
| 16 - Avg. of objects Avg Intensity; | 16 - STD of 15 |
| 18 - Avg. of objects Median intensity; | 19 - STD of 18 |
| 20 - Avg. of objects intensity of 20% bright pixel; | 21 - STD of 19 |
| 22 - Avg. of objects intensity of 80% bright pixel; | 23 - STD of 21 |

An example of summary output obtained by running AnalyseDNA against 10 montage files also is shown in Appendix B.

10 A seventh experiment was conducted in order to use sequence analysis algorithms to analyze features of cell images. In this experiment, HeLa cells were treated for 24 hours with several different compounds, and then fixed, and stained with a fluorescent DNA dye. One image of these cells was acquired for each of the treatments and morphometric parameters and features were measured:

15 Resulting measurements were arranged into a string of numbers and reduced to a pseudo- nucleic acid sequence using following rules: At any given position in the sequence a number was substituted by "t" (a code for thymidine) if its value is among highest 25% of the values at the corresponding position in the data set, "g" if it is between 50% and 25%, "c" if it is between 75% and 50%, and "a" if it
20 belongs to lowest 25% of values. Thus one descriptor or sequence was generated per treatment as illustrated in Fig. 18.

Resulting sequences were clustered using an AlignX module commercial software package Vector NTI (<http://informaxinc.com>), which uses a Neighbor Joining algorithm for sequence clustering.

25 The resulting dendrogram is presented in Fig 18. On the dendrogram the closest "leafs" correspond to the closest pseudo-sequences. Interestingly, compounds with similar mechanisms of action cluster together on the dendrogram. Another example of the generation of pseudo-sequences and clustering is shown in Fig. 19.

30 In some embodiments, techniques according to the present invention can provide tools for the later stages of drug development such as clinical trial design and patient management. The properties of known drugs such as clinical trial and patient response information will be used in a similar fashion as the pre-clinical

information to provide predictions about the properties of novel compounds. Because the human cell is the locus of drug action, a database containing drug-cell interactions can be able to provide predictive information for this aspect of drug development.

Although the above has generally described the present invention

5 according to specific systems, the present invention has a much broader range of applicability. In particular, the present invention is not limited to a particular kind of data about a cell, but can be applied to virtually any cellular data where an understanding about the workings of the cell is desired. Thus, in some embodiments, the techniques of the present invention could provide information about many

10 different types or groups of cells, substances, and genetic processes of all kinds. Of course, one of ordinary skill in the art would recognize other variations, modifications, and alternatives.

Appendix A

EV Table 1.doc

Example of the output of AnalyseDNA.m program
(measurements for a single 3 by 3 montage image)

File#	Subimage	object#	X coord.	Y coord.	Area	Arm ratio	Eccentri- city	Equival- ent	Solidity	Extent	Intensi- ty	Avg. Intensi- ty	Median Intensi- ty	20% pla.	80% pla.
1	1	1	12.2897	152.655	115	1.17291	0.322614	13.5875	0.933567	0.739796	4605	31.7386	34	23	37
2	2	1	16.352	416.032	125	1.40594	0.382471	12.6157	0.905391	0.78125	4606	36.848	31	30	45
3	3	1	20.1073	72.8039	137	1.09845	0.413785	15.0121	0.917098	0.681026	4609	26.9435	29	27	31
4	4	1	21.684	407.144	43	1.16215	0.418004	11.0928	0.914894	0.767857	3690	85.814	87	67	105
5	5	1	21.0316	181.534	96	1.10887	0.445194	11.0558	0.888889	0.671329	4502	46.8958	49	38	56
6	6	1	30.7352	359.534	206	2.23106	0.401209	16.1953	0.927978	0.915278	6380	30.9709	31	24	37
7	7	1	32.6279	167.537	89	1.34984	0.471694	10.4451	0.932083	0.711667	4725	47.4119	50	39	56
8	8	1	32.6279	167.537	146	1.25176	0.401495	13.6143	0.929936	0.718018	5115	37.089	40	29	44
9	9	1	32.6279	167.537	146	1.25176	0.401495	13.6143	0.929936	0.718018	5115	37.089	40	29	44
10	10	1	49.1078	344.031	43	1.40462	0.419542	11.7159	0.91037	0.632778	4667	141.851	162	113	171
11	11	1	49.1078	344.031	232	1.90491	0.451127	11.187	0.852941	0.707003	5872	47.3793	65	33	51
12	12	1	56.0749	176.534	271	1.51704	0.455555	16.7146	0.924686	0.686335	7040	31.8552	33	25	37
13	13	1	52.7755	41.9332	171	1.51673	0.463701	13.6009	0.907409	0.706731	4745	32.415	34	26	39
14	14	1	52.7755	41.9332	171	1.51673	0.463701	13.6009	0.907409	0.706731	4745	32.415	34	26	39
15	15	1	56.4079	346.854	371	2.73725	0.454944	16.3953	0.923367	0.706612	9318	56.8121	56	43	68
16	16	1	57.0648	277.116	206	1.71883	0.418088	13.3265	0.915254	0.701799	4644	31.6156	37	28	41
17	17	1	61.1714	333.181	315	1.31194	0.432766	10.0567	0.75	0.526256	13151	48.0864	50	36	62
18	18	1	61.1714	333.181	315	1.31194	0.432766	10.0567	0.75	0.526256	13151	48.0864	50	36	62
19	19	1	65.1409	402.411	270	1.70167	0.409064	16.7166	0.920502	0.641059	9809	44.5864	46	35	54
20	20	1	65.1409	402.411	270	1.70167	0.409064	16.7166	0.920502	0.641059	9809	44.5864	46	35	54
21	21	1	73.4849	443.13	185	1.71588	0.418422	12.5162	0.911111	0.732528	4841	39.3577	41	30	47
22	22	1	73.4849	443.13	185	1.71588	0.418422	12.5162	0.911111	0.732528	4841	39.3577	41	30	47
23	23	1	78.7377	208.27	172	1.3157	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
24	24	1	81.4786	51.5912	117	1.44513	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
25	25	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
26	26	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
27	27	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
28	28	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
29	29	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
30	30	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
31	31	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
32	32	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
33	33	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
34	34	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
35	35	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
36	36	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
37	37	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
38	38	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
39	39	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
40	40	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
41	41	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
42	42	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
43	43	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
44	44	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
45	45	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
46	46	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
47	47	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
48	48	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
49	49	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
50	50	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
51	51	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
52	52	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
53	53	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
54	54	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
55	55	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
56	56	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
57	57	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
58	58	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
59	59	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
60	60	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
61	61	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
62	62	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
63	63	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
64	64	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
65	65	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
66	66	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
67	67	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47
68	68	1	88.1792	281.534	373	2.17388	0.467941	12.6414	0.923581	0.732528	4841	39.3577	41	30	47

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1	1	217.509	86.7657	224	1.87991	0.846782	16.888	0.899598	0.532364	8822	39.4063	42	30
2	1	218.831	322.146	160	1.79661	0.830818	14.273	0.91956	0.74535	5025	31.063	33	25
3	1	219.214	413.076	77	1.51646	0.498531	9.90149	0.915375	0.7	4332	56.3195	59	46
4	1	220.945	43.816	163	1.56853	0.777002	11.4062	0.915375	0.7	4770	26.5571	30	23
5	1	221.601	398.848	66	1.03149	0.745208	9.167	0.916667	0.879167	4770	43.7277	71	56
6	1	223.601	375.38	251	1.95991	0.840038	17.8768	0.866326	0.597619	10500	41.7371	33	32
7	1	224.931	237.401	161	1.45084	0.795632	14.3175	0.914733	0.888034	5176	11.8066	33	25
8	1	226.931	209.402	111	1.31512	0.649722	11.8882	0.917355	0.720759	4858	33.7658	45	36
9	1	228.931	348.328	131	1.58165	0.774929	12.8159	0.909722	0.882292	9623	37.5284	77	56
10	1	230.931	371.324	204	2.05613	0.873763	18.1165	0.918519	0.596691	7051	24.2537	35	28
11	1	232.931	285.098	287	1.27832	0.627825	19.316	0.87274	0.650391	10550	38.4531	78	45
12	1	234.931	372.32	150	1.40321	0.672329	13.8186	0.920245	0.765304	9202	41.3167	63	42
13	1	236.931	391.118	85	1.47318	0.801767	10.4031	0.923913	0.817308	4397	51.4118	55	41
14	1	238.931	354.319	211	1.50101	0.74855	17.1499	0.931632	0.675639	6580	27.1479	59	31
15	1	240.931	203.488	221	1.75168	0.872092	16.7746	0.882883	0.701597	10251	48.3846	69	51
16	1	242.931	355.072	66	1.71017	0.810394	7.65708	0.881923	0.730159	6996	151.87	159	120
17	1	244.931	319.71	182	1.31164	0.618661	13.5875	0.917722	0.735208	5910	31.0442	32	27
18	1	246.931	442.714	58	2.01531	0.869208	15.4533	0.911176	0.791388	5972	31.1042	32	27
19	1	248.931	349.714	192	1.25774	0.695112	14.2283	0.928235	0.757143	5103	62.3043	70	55
20	1	250.931	285.162	159	1.39289	0.69663	13.8166	0.925976	0.78125	3159	35.7933	37	30
21	1	252.931	358.167	150	1.35157	0.727432	22.054	0.913267	0.598746	16117	42.1911	44	32
22	1	254.931	258.38	392	1.35663	0.69165	14.3175	0.916067	0.764667	4866	30.8447	33	24
23	1	256.931	312.894	181	1.30863	0.789304	15.1788	0.937642	0.728745	4915	27.3056	28	27
24	1	258.931	372.894	111	1.48164	0.791615	12.466	0.9	0.7	4950	39.2857	60	31
25	1	260.931	204.518	126	1.40033	0.84651	13.4198	0.887353	0.555728	4958	33.0533	35	25
26	1	262.931	43.08	150	2.0034	0.84651	13.4198	0.917722	0.74359	5048	32.8138	36	28
27	1	264.931	322.468	145	1.3164	0.565162	12.5875	0.917722	0.74359	5048	32.8138	36	28
28	1	266.931	227.468	145	1.3164	0.565162	12.5875	0.917722	0.74359	5048	32.8138	36	28
29	1	268.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
30	1	270.931	356.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
31	1	272.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
32	1	274.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
33	1	276.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
34	1	278.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
35	1	280.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
36	1	282.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
37	1	284.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
38	1	286.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
39	1	288.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
40	1	290.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
41	1	292.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
42	1	294.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
43	1	296.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
44	1	298.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
45	1	300.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
46	1	302.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
47	1	304.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
48	1	306.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
49	1	308.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
50	1	310.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
51	1	312.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
52	1	314.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
53	1	316.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
54	1	318.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
55	1	320.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
56	1	322.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
57	1	324.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
58	1	326.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
59	1	328.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
60	1	330.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
61	1	332.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
62	1	334.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
63	1	336.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
64	1	338.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
65	1	340.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
66	1	342.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
67	1	344.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
68	1	346.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
69	1	348.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
70	1	350.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
71	1	352.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
72	1	354.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
73	1	356.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
74	1	358.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
75	1	360.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
76	1	362.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
77	1	364.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
78	1	366.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
79	1	368.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
80	1	370.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
81	1	372.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
82	1	374.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
83	1	376.931	396.018	147	1.75168	0.872092	16.7746	0.916306	0.771646	9195	42.551	65	47
84	1	378.931	396.018	147	1.75168	0.8720							

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1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	537	538	539	540	541	542	543	544	545	546	547	548	549	550	551	552	553	554	555	556	557	558	559	560	561	562	563	564	565	566	567	568	569	570	571	572	573	574	575	576	577	578	579	580	581	582	583	584	585	586	587	588	589	590	591	592	593	594	595	596	597	598	599	600	601	602	603	604	605	606	607	608	609	610	611	612	613	614	615	616	617	618	619	620	621	622	623	624	625	626	627	628	629	630	631	632	633	634	635	636	637	638	639	640	641	642	643	644	645	646	647	648	649	650	651	652	653	654	655	656	657	658	659	660	661	662	663	664	665	666	667	668	669	670	671	672	673	674	675	676	677	678	679	680	681	682	683	684	685	686	687	688	689	690	691	692	693	694	695	696	697	698	699	700	701	702	703	704	705	706	707	708	709	710	711	712	713	714	715	716	717	718	719	720	721	722	723	724	725	726	727	728	729	730	731	732	733	734	735	736	737	738	739	740	741	742	743	744	745	746	747	748	749	750	751	752	753	754	755	756	757	758	759	760	761	762	763	764	765	766	767	768	769	770	771	772	773	774	775	776	777	778	779	780	781	782	783	784	785	786	787	788	789	790	791	792	793	794	795	796	797	798	799	800	801	802	803	804	805	806	807	808	809	810	811	812	813	814	815	816	817	818	819	820	821	822	823	824	825	826	827	828	829	830	831	832	833	834	835	836	837	838	839	840	841	842	843	844	845	846	847	848	849	850	851	852	853	854	855	856	857	858	859	860	861	862	863	864	865	866	867	868	869	870	871	872	873	874	875	876	877	878	879	880	881	882	883	884	885	886	887	888	889	890	891	892	893	894	895	896	897	898	899	900	901	902	903	904	905	906	907	908	909	910	911	912	913	914	915	916	917	918	919	920	921	922	923	924	925	926	927	928	929	930	931	932	933	934	935	936	937	938	939	940	941	942	943	944	945	946	947	948	949	950	951	952	953	954	955	956	957	958	959	960	961	962	963	964	965	966	967	968	969	970	971	972	973	974	975	976	977	978	979	980	981	982	983	984	985	986	987	988	989	990	991	992	993	994	995	996	997	998	999	1000
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1	1	136	483.48	366.419	322	2.80072	0.931053	19.5115	0.89521	0.48894	1.065	47.0001	50	38	57
2	2	137	488.732	368.763	323	1.75129	0.921058	15.5316	0.835156	0.666667	8186	46.2421	50	35	57
3	3	138	492.318	371.267	324	1.4249	0.913058	15.731	0.813014	0.713081	5186	47.3133	29	33	51
4	4	139	495.318	374.018	325	1.21521	0.905157	11.1361	0.808119	0.713747	5508	54	38	64	
5	5	140	498.318	376.515	326	1.09922	0.900152	11.609	0.800214	0.714671	5098	34.4811	37	25	44
6	6	141	501.318	379.018	327	1.00016	0.895154	16.966	0.795116	0.617059	9866	56.0549	60	45	68
7	7	142	504.318	381.515	328	1.00016	0.890154	10.2119	0.881172	0.683333	4420	53.9028	56	46	68
8	8	143	507.318	384.018	329	1.00016	0.885154	16.3097	0.876176	0.701333	9318	45.2038	47	35	55
9	9	144	510.318	386.515	330	1.00016	0.880154	16.3097	0.871176	0.717476	4079	55.1216	56	46	68
10	10	145	513.318	389.018	331	1.00016	0.875154	16.3097	0.866176	0.733619	4079	37.7216	58	48	70
11	11	146	516.318	391.515	332	1.00016	0.870154	16.3097	0.861176	0.749562	3382	39.6148	61	51	73
12	12	147	519.318	394.018	333	1.00016	0.865154	16.3097	0.856176	0.765505	3382	31.529	33	24	39
13	13	148	522.318	396.515	334	1.00016	0.860154	16.3097	0.851176	0.781448	3382	40.2103	42	31	50
14	14	149	525.318	399.018	335	1.00016	0.855154	16.3097	0.846176	0.797391	3382	37.3152	36	27	42
15	15	150	528.318	401.515	336	1.00016	0.850154	16.3097	0.841176	0.813333	3382	44.5616	46	34	53
16	16	151	531.318	404.018	337	1.00016	0.845154	16.3097	0.836176	0.829276	3382	35.1655	36	27	42
17	17	152	534.318	406.515	338	1.00016	0.840154	16.3097	0.831176	0.845219	3382	41.8162	46	34	53
18	18	153	537.318	409.018	339	1.00016	0.835154	16.3097	0.826176	0.861162	3382	38.4815	40	30	46
19	19	154	540.318	411.515	340	1.00016	0.830154	16.3097	0.821176	0.877105	3382	34.7861	36	27	42
20	20	155	543.318	414.018	341	1.00016	0.825154	16.3097	0.816176	0.893048	3382	63.8512	69	52	74
21	21	156	546.318	416.515	342	1.00016	0.820154	16.3097	0.811176	0.908991	3382	39.4015	36	27	42
22	22	157	549.318	419.018	343	1.00016	0.815154	16.3097	0.806176	0.924934	3382	34.9244	36	27	42
23	23	158	552.318	421.515	344	1.00016	0.810154	16.3097	0.801176	0.940877	3382	47.9162	46	34	53
24	24	159	555.318	424.018	345	1.00016	0.805154	16.3097	0.796176	0.956820	3382	44.1104	46	34	53
25	25	160	558.318	426.515	346	1.00016	0.800154	16.3097	0.791176	0.972763	3382	32.3125	34	26	38
26	26	161	561.318	429.018	347	1.00016	0.795154	16.3097	0.786176	0.988706	3382	31.2911	33	25	37
27	27	162	564.318	431.515	348	1.00016	0.790154	16.3097	0.781176	0.994649	3382	72.5222	74	53	89
28	28	163	567.318	434.018	349	1.00016	0.785154	16.3097	0.776176	0.994649	3382	35.9101	33	29	43
29	29	164	570.318	436.515	350	1.00016	0.780154	16.3097	0.771176	0.994649	3382	34.2116	36	26	42
30	30	165	573.318	439.018	351	1.00016	0.775154	16.3097	0.766176	0.994649	3382	43.4081	46	34	53
31	31	166	576.318	441.515	352	1.00016	0.770154	16.3097	0.761176	0.994649	3382	37.9108	40	30	46
32	32	167	579.318	444.018	353	1.00016	0.765154	16.3097	0.756176	0.994649	3382	38.436	39	29	48
33	33	168	582.318	446.515	354	1.00016	0.760154	16.3097	0.751176	0.994649	3382	36.3134	40	31	46
34	34	169	585.318	449.018	355	1.00016	0.755154	16.3097	0.746176	0.994649	3382	65.1405	70	51	81
35	35	170	588.318	451.515	356	1.00016	0.750154	16.3097	0.741176	0.994649	3382	30.8658	33	25	36
36	36	171	591.318	454.018	357	1.00016	0.745154	16.3097	0.736176	0.994649	3382	30.8658	33	25	36
37	37	172	594.318	456.515	358	1.00016	0.740154	16.3097	0.731176	0.994649	3382	30.8658	33	25	36
38	38	173	597.318	459.018	359	1.00016	0.735154	16.3097	0.726176	0.994649	3382	30.8658	33	25	36
39	39	174	600.318	461.515	360	1.00016	0.730154	16.3097	0.721176	0.994649	3382	30.8658	33	25	36
40	40	175	603.318	464.018	361	1.00016	0.725154	16.3097	0.716176	0.994649	3382	30.8658	33	25	36
41	41	176	606.318	466.515	362	1.00016	0.720154	16.3097	0.711176	0.994649	3382	30.8658	33	25	36
42	42	177	609.318	469.018	363	1.00016	0.715154	16.3097	0.706176	0.994649	3382	30.8658	33	25	36
43	43	178	612.318	471.515	364	1.00016	0.710154	16.3097	0.701176	0.994649	3382	30.8658	33	25	36
44	44	179	615.318	474.018	365	1.00016	0.705154	16.3097	0.696176	0.994649	3382	30.8658	33	25	36
45	45	180	618.318	476.515	366	1.00016	0.700154	16.3097	0.691176	0.994649	3382	30.8658	33	25	36
46	46	181	621.318	479.018	367	1.00016	0.695154	16.3097	0.686176	0.994649	3382	30.8658	33	25	36
47	47	182	624.318	481.515	368	1.00016	0.690154	16.3097	0.681176	0.994649	3382	30.8658	33	25	36
48	48	183	627.318	484.018	369	1.00016	0.685154	16.3097	0.676176	0.994649	3382	30.8658	33	25	36
49	49	184	630.318	486.515	370	1.00016	0.680154	16.3097	0.671176	0.994649	3382	30.8658	33	25	36
50	50	185	633.318	489.018	371	1.00016	0.675154	16.3097	0.666176	0.994649	3382	30.8658	33	25	36
51	51	186	636.318	491.515	372	1.00016	0.670154	16.3097	0.661176	0.994649	3382	30.8658	33	25	36
52	52	187	639.318	494.018	373	1.00016	0.665154	16.3097	0.656176	0.994649	3382	30.8658	33	25	36
53	53	188	642.318	496.515	374	1.00016	0.660154	16.3097	0.651176	0.994649	3382	30.8658	33	25	36
54	54	189	645.318	499.018	375	1.00016	0.655154	16.3097	0.646176	0.994649	3382	30.8658	33	25	36
55	55	190	648.318	501.515	376	1.00016	0.650154	16.3097	0.641176	0.994649	3382	30.8658	33	25	36
56	56	191	651.318	504.018	377	1.00016	0.645154	16.3097	0.636176	0.994649	3382	30.8658	33	25	36
57	57	192	654.318	506.515	378	1.00016	0.640154	16.3097	0.631176	0.994649	3382	30.8658	33	25	36
58	58	193	657.318	509.018	379	1.00016	0.635154	16.3097	0.626176	0.994649	3382	30.8658	33	25	36
59	59	194	660.318	511.515	380	1.00016	0.630154	16.3097	0.621176	0.994649	3382	30.8658	33	25	36
60	60	195	663.318	514.018	381	1.00016	0.625154	16.3097	0.616176	0.994649	3382	30.8658	33	25	36
61	61	196	666.318	516.515	382	1.00016	0.620154	16.3097	0.611176	0.994649	3382	30.8658	33	25	36
62	62	197	669.318	519.018	383	1.00016	0.615154	16.3097	0.606176	0.994649	3382	30.8658	33	25	36
63	63	198	672.318	521.515	384	1.00016	0.610154	16.3097	0.601176	0.994649	3382	30.8658	33	25	36
64	64	199	675.318	524.018	385	1.00016	0.605154	16.3097	0.596176	0.994649	3382	30.8658	33	25	36
65	65	200	678.318	526.515	386	1.00016	0.600154	16.3097	0.591176	0.994649	3382	30.8658	33	25	36
66	66	201	681.318	529.018	387	1.00016	0.595154	16.3097	0.586176	0.994649	3382	30.8658	33	25	36
67	67	202	684.318	531.515	388	1.00016	0.590154	16.3097	0.581176	0.994649	3382	30.8658	33	25	36
68	68	203	687.318	534.018	389	1.00016	0.585154	16.3097	0.576176	0.994649	3382	30.8658	33	25	36
69	69	204	690.318	536.515	390	1.00016	0.580154	16.3097	0.571176	0.994649	3382	30.8658	33	25	36
70	70	205	693.318	539.018	391	1.00016	0.575154	16.3097	0.566176	0.994649	3382	30.8658	33	25	36
71	71	206	696.318	541.515	392	1.00016	0.570154	16.3097	0.561176	0.994649	3382	30.8658	33	25	36
72	72	207	699.318	544.018	393	1.00016	0.565154	16.3097	0.556176	0.994649	3382	30.8658	33	25	36
73	73	208	702.318	546.515	394	1.00016	0.560154	16.3097	0.551176	0.994649	3382	30.8658	33	25	36
74	74	209	705.318	549.018	395	1.00016	0.555154	16.3097	0.546176	0.994649	3382	30.8658	33	25	36
75	75	210	708.318	551.515	396	1.00016	0.550154	16.3097	0.541176	0.994649	3382	30.8658			

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1	1	270.971	433.637	177	1.27886	0.624112	15.0171	0.917058	0.743657	9749	55.0191	55	69
2	2	273.484	472.243	221	1.27886	0.815564	16.7166	0.884	0.618889	9512	43.3172	45	51
3	3	275.832	515.815	208	2.57259	0.91874	16.7237	0.808297	0.619048	9810	47.1635	46	51
4	4	278.911	595.184	408	2.04161	0.872635	21.9267	0.741661	0.510435	15133	31.0102	37	24
5	5	282.431	700.619	144	1.66644	0.844395	13.5066	0.9	0.666667	1897	56.3547	56	42
6	6	288.341	756.004	249	2.49369	0.928094	17.8055	0.778175	0.523109	10866	42.1124	43	33
7	7	290.055	492.603	189	1.27162	0.848177	15.5176	0.921851	0.75	6743	25.0952	21	20
8	8	292.768	233.6071	312	1.45137	0.741889	11.9116	0.888889	0.622722	4862	41.3371	47	24
9	9	295.44	719.131	293	1.2724	0.531525	19.3117	0.931299	0.827278	13163	45.9408	47	34
10	10	296.917	64.0877	134	1.33891	0.759372	12.0478	0.80625	0.60809	4599	40.2344	40	34
11	11	298.141	204.621	121	1.31011	0.659521	12.5651	0.939394	0.826667	6013	48.9191	51	34
12	12	298.431	318.43	129	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
13	13	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
14	14	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
15	15	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
16	16	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
17	17	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
18	18	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
19	19	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
20	20	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
21	21	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
22	22	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
23	23	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
24	24	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
25	25	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
26	26	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
27	27	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
28	28	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
29	29	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
30	30	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
31	31	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
32	32	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
33	33	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
34	34	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
35	35	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
36	36	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
37	37	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
38	38	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
39	39	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
40	40	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
41	41	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
42	42	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
43	43	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
44	44	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
45	45	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
46	46	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
47	47	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
48	48	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
49	49	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
50	50	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
51	51	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
52	52	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
53	53	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
54	54	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
55	55	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
56	56	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
57	57	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
58	58	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
59	59	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
60	60	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
61	61	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
62	62	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
63	63	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
64	64	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
65	65	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
66	66	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
67	67	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
68	68	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
69	69	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
70	70	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
71	71	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
72	72	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
73	73	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
74	74	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
75	75	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
76	76	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
77	77	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
78	78	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
79	79	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
80	80	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
81	81	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
82	82	298.431	327.056	133	1.32744	0.459521	12.8159	0.902049	0.716667	6258	48.5116	51	39
83	83	298.431	327.056	133	1								

EV Table 1.doc	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200
	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300
	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400
	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500
	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519</																																																																																	

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EV Table 1.doc

1	1	3	246.803	66.7186	334	1.70555	0.810104	20.4219	0.885942	0.703158	15594	46.988	49	38	55
2	1	3	246.436	115.889	165	1.57535	0.712771	13.3875	0.917732	0.697115	4689	32.2379	33	75	39
3	1	3	245.743	266.532	173	1.45335	0.725714	14.8815	0.905159	0.686508	9292	51.711	56	44	64
4	1	3	245.645	297.404	148	1.76551	0.819627	13.7273	0.907975	0.686683	6158	41.6081	44	37	50
5	1	3	245.892	35.5	166	1.69253	0.800796	15.389	0.925373	0.615833	7714	41.4731	44	34	50
6	1	3	247.611	474.84	288	1.69718	0.800108	15.1492	0.953143	0.626087	12451	43.7326	45	33	53
7	1	3	246.033	256.171	152	1.66024	0.798253	13.9116	0.91018	0.703708	6365	41.875	44	34	49
8	1	3	248.878	681.764	173	1.8018	0.831849	12.5143	0.91791	0.803922	4486	36.4715	38	29	43
9	1	3	295.075	642.753	166	1.6836	0.805009	12.6143	0.918239	0.737276	5724	39.2055	40	30	46
10	1	3	300.644	678.541	127	1.79398	0.810231	12.4634	0.917293	0.797186	4889	36.1951	39	30	43
11	1	3	316.606	113.728	254	1.95525	0.859316	17.9834	0.930665	0.574661	10021	39.4528	42	31	47
12	1	3	316.636	410.356	131	1.27616	0.621246	12.9149	0.89326	0.668167	7819	60.145	63	48	73
13	1	3	334.614	618.425	191	1.46831	0.732318	22.3123	0.907851	0.55649	18141	47.9208	47	36	47
14	1	3	334.399	87.0629	143	1.88512	0.847704	17.4915	0.910828	0.752632	14531	37.5185	34	27	34
15	1	3	346.491	100.553	219	2.21562	0.892351	16.6965	0.943964	0.51664	5193	21.7123	24	19	24
16	1	3	346.328	642.77	174	1.41208	0.766034	14.8813	0.925484	0.725	6167	34.597	37	32	44
17	1	3	356.316	138.137	232	1.8771	0.848052	17.387	0.939271	0.659091	3191	22.375	24	18	27
18	1	3	353.332	178.753	265	1.66527	0.798621	19.3687	0.886288	0.755841	11544	41.5823	45	36	52
19	1	3	348.601	52.3114	228	1.73712	0.817484	17.0382	0.919255	0.623333	9210	41.3991	43	33	54
20	1	3	372.972	19.3934	273	1.41274	0.701149	14.4582	0.938225	0.747168	9210	43.2394	44	32	54
21	1	3	374.753	117.607	150	1.29951	0.638622	13.8198	0.943196	0.78125	4817	32.5133	34	27	38
22	1	3	374.303	311.178	152	1.15034	0.494272	13.9116	0.921212	0.71551	4803	31.625	32	24	38
23	1	3	377.8	376.522	135	1.45456	0.724189	12.1005	0.912488	0.731179	4432	38.5824	40	30	46
24	1	3	378.921	365.124	246	1.81817	0.841264	18.4033	0.896312	0.731889	9883	37.1541	39	29	44
25	1	3	384.356	250.143	220	2.04768	0.872617	17.3322	0.916325	0.525	7281	24.0003	38	29	44
26	1	3	385.292	492.842	202	1.46285	0.84372	15.0233	0.915185	0.765152	8562	42.4109	45	34	51
27	1	3	410.504	429.717	138	1.74883	0.810613	13.7034	0.904497	0.720536	6281	30.8705	32	25	36
28	1	3	411.49	466.48	258	1.41752	0.700752	18.1595	0.845355	0.700933	9624	37.184	38	30	45
29	1	3	422.424	135.104	238	1.81888	0.815053	20.1594	0.81582	0.585134	10529	31.7957	35	25	38
30	1	3	423.127	154.422	333	1.41841	0.784244	21.2001	0.827864	0.617814	12182	41.6384	41	31	45
31	1	3	440.34	90.4832	203	1.71659	0.841832	18.0983	0.892738	0.701189	7071	31.5099	36	25	34
32	1	3	454.719	235.656	251	2.92458	0.881182	16.0983	0.855312	0.697231	7289	31.2497	33	23	45
33	1	3	452.566	444.405	136	1.58197	0.779014	13.1531	0.925312	0.697231	7289	31.2497	33	23	45
34	1	3	464.412	246.418	284	1.22485	0.652225	13.353	0.90508	0.604894	15055	52.384	55	40	63
35	1	3	468.914	244.433	326	1.42791	0.789625	20.3777	0.915371	0.686316	13203	55.8374	58	45	66
36	1	3	468.914	244.433	326	1.42791	0.789625	20.3777	0.915371	0.686316	13203	55.8374	58	45	66
37	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
38	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
39	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
40	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
41	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
42	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
43	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
44	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
45	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
46	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
47	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
48	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
49	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
50	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
51	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
52	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
53	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
54	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
55	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
56	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
57	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
58	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
59	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
60	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
61	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
62	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
63	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
64	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
65	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
66	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
67	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
68	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
69	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
70	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
71	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
72	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
73	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
74	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
75	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
76	1	3	471.127	211.104	251	1.9117	0.855375	11.0558	0.905164	0.623777	2616	37.6667	39	29	44
77	1	3	471.127	211.104	251	1.9117	0.855375	1							

20	132.584	670.015	750	1.71836	0.813219	17.8432	0.928368	0.657895	5098	20.392	21	16
21	134.597	731.276	155	1.36192	0.662359	12.1005	0.894673	0.704967	4161	36.1636	38	28
22	136.597	771.340	135	1.70683	0.810918	12.9573	0.910716	0.708333	4600	28.7582	29	27
23	138.597	781.081	99	1.54808	0.750701	11.2772	0.9	0.692308	4268	63.1113	45	35
24	135.642	776.505	196	1.48408	0.804616	15.1973	0.806584	0.600523	5941	69.1148	49	38
25	136.281	755.452	171	1.40236	0.78167	14.7555	0.805576	0.616571	5972	23.1017	74	26
26	135.941	732	118	1.66552	0.866181	12.2573	0.914778	0.670555	4024	22.4155	78	28
27	143.395	60.8098	205	2.46837	0.915523	16.1559	0.921623	0.716193	4594	37.5277	75	28
28	136.271	697.708	107	2.61388	0.923925	11.672	0.89916	0.629412	6016	37.5277	75	28
29	141.468	246.271	181	1.38148	0.689166	15.1608	0.918782	0.708801	8158	48.2991	61	37
30	151.392	60.6022	181	1.20958	0.562546	15.1608	0.918782	0.708801	8158	48.2991	61	37
31	155.006	138.468	173	1.57106	0.791716	14.8415	0.910526	0.720833	7160	44.2775	63	35
32	158.17	371.076	105	1.53163	0.731169	11.5674	0.913753	0.722727	6553	31.1333	55	32
33	158.17	311.515	159	1.62167	0.83658	14.7281	0.911793	0.722727	6553	28.0063	79	31
34	163.166	62.5155	97	1.30584	0.643091	11.1132	0.92381	0.746516	4091	42.1753	55	32
35	163.166	193.375	73	1.19543	0.541917	9.40088	0.879516	0.645164	2333	31.5988	37	24
36	164.629	68.973	105	1.30539	0.642734	11.5674	0.805172	0.732766	4094	39.0053	60	40
37	168.383	77.1158	81	1.09868	0.410546	10.1554	0.89011	0.716364	3980	49.1158	50	40
38	171.28	61.194	91	1.61821	0.738156	10.8817	0.93	0.704945	3451	37.1013	59	30
39	171.28	405.46	124	1.15373	0.67943	12.1662	0.933333	0.807692	3872	20.7702	37	25
40	171.594	492.432	128	1.15373	0.67943	12.1662	0.933333	0.807692	3872	20.7702	37	25
41	173.67	222.276	201	1.35599	0.873539	14.0769	0.931193	0.751552	8886	42.2466	16	31
42	173.67	331.715	102	1.61242	0.8301	11.3861	0.87931	0.60355	4789	42.049	43	31
43	184.008	66.8987	147	2.74657	0.931981	17.3712	0.927642	0.50641	8974	27.5152	78	21
44	194.927	300.864	232	1.61442	0.836412	14.5819	0.936616	0.77512	4657	34.847	37	28
45	196.448	323.525	282	1.85598	0.842417	19.9187	0.881735	0.582645	5926	34.847	37	28
46	198.333	198.169	64	1.37558	0.68779	9.37302	0.807895	0.784091	2189	31.7746	37	28
47	198.333	410.946	142	1.36735	0.693144	13.4642	0.810258	0.682692	4317	30.4014	31	26
48	198.333	15.0249	172	1.53588	0.758984	14.7886	0.912798	0.781875	8151	48.1333	51	30
49	203.48	182.972	103	1.19441								

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106	355.018	312.36	1.76044	0.877402	12.0078	0.897698	0.878374	4181	36.4754	39	29	43
107	361.714	169.031	1.40115	0.781048	12.8159	0.908451	0.661538	1538	58.4186	60	46	46
108	370.472	126.786	1.37746	0.46247	11.9416	0.903226	0.717609	4218	37.6607	40	30	46
109	379.866	101.258	1.21537	0.568327	11.1132	0.898148	0.731888	4148	42.7216	48	34	51
110	382.737	601.684	1.14859	0.800559	15.4104	0.912195	0.611111	6530	48.1487	48	36	51
111	388.96	193.188	1.14859	0.815133	19.6415	0.907198	0.613125	11210	38.9967	39	29	45
112	392.155	331.273	1.14859	0.815133	19.6415	0.915189	0.725	8357	50.3276	52	39	61
113	394.888	241.441	1.14859	0.815133	19.6415	0.947733	0.653166	4577	37.6544	35	27	41
114	401.428	302.432	1.14859	0.815133	19.6415	0.865109	0.613333	19684	41.3458	50	32	41
115	399.517	232.316	2.04191	0.877402	12.0078	0.925166	0.616753	5111	36.3154	38	29	44
116	416.693	273.039	1.50902	0.748801	12.2193	0.940878	0.610101	4592	26.0266	27	20	32
117	424.436	30.7862	1.63114	0.740103	14.2293	0.916324	0.762238	3503	35.8005	29	22	35
118	429	16	1.24376	0.584169	11.7808	0.921077	0.713333	4152	31.4545	33	25	38
119	431.508	178.795	1.38334	0.680866	12.9441	0.951807	0.603233	10217	25.8638	24	20	31
120	422.648	371.2	1.68431	0.80468	22.4261	0.951807	0.603233	10217	31.3626	43	33	51
121	435.648	108.709	1.41734	0.713551	10.7411	0.91	0.727778	3764	37.7023	34	25	39
122	439.949	245.441	1.74057	0.816888	12.9149	0.891156	0.727778	3764	38.4091	40	29	47
123	441.145	472.391	1.52612	0.755407	11.8145	0.927228	0.651713	4268	25.2497	27	20	30
124	447.904	87.8989	1.58428	0.755407	15.0545	0.941667	0.601163	4165	38.4104	39	30	44
125	450.265	488.54	1.37622	0.686912	11.9948	0.904878	0.708232	3858	38.3164	34	29	44
126	451.785	140.813	1.23762	0.604412	11.672	0.904878	0.708232	3858	38.3164	34	29	44
127	456.12	159.5	1.2749	0.620284	11.2038	0.925976	0.718734	4147	21.2822	33	24	38
128	461.271	117.674	1.7004	0.808791	17.0131	0.875	0.537324	4147	36.6537	38	28	46
129	463.58	188.899	1.7076	0.810637	18.0693	0.911148	0.453516	4080	80	79	53	98
130	472.742	297.325	1.12911	0.646354	11.3201	0.90951	0.715152	4309	29.3172	30	23	36
131	477.607	338.876	1.23853	0.589592	13.5975	0.927567	0.715152	4309	34.5259	39	30	43
132	477.543	342.586	1.10393	0.673593	12.153	0.913386	0.715152	4309	39.1743	60	31	49
133	479.45	22.1651	1.42045	0.710199	11.7806	0.927728	0.718734	4270	30.3581	33	25	37
134	485.604	60.2073	2.07652	0.876405	11.4503	0.914701	0.718734	4270	102.319	109	85	134
135	480.397	166.805	1.41513	0.707564	15.9573	0.951151	0.641	4343	79.3116	41	31	47
136	480.58	251.707	1.53736	0.766838	14.8943	0.935481	0.67159	3885	62.7803	63	51	75
137	487.806	313.887	1.60841	0.793231	18.8497	0.949551	0.67159	3885	42.7019	41	33	50
138	495.029	311.5	2.02048	0.678165	16.2317	0.927131	0.67159	3885	41.9192	41	33	50
139	498.219	7.92709	1.10584	0.677089	11.0558	0.905814	0.67159	3885	36.5555	38	29	44
140	501.108	502.892	1.00155	0.551714	12.1005	0.920192	0.67159	3885	38.788	40	32	46
141	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
142	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
143	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
144	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
145	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
146	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
147	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
148	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
149	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
150	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
151	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
152	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
153	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
154	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
155	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
156	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
157	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
158	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
159	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
160	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
161	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
162	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
163	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
164	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
165	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
166	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
167	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
168	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
169	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
170	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
171	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
172	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
173	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
174	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
175	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
176	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
177	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
178	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
179	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
180	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
181	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
182	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
183	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
184	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
185	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
186	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
187	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
188	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
189	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
190	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
191	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42	32	47
192	501.108	502.892	1.00155	0.551714	12.1005	0.931892	0.67159	3885	40.1837	42		

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1	11.1154	387.547	104	1.42586	0.71237	11.5073	0.504346	0.673325	3955	39.0288	39	31	56
2	9.40303	429.712	68	1.20485	0.518881	9.167	0.88	0.713333	3059	66.3485	48	40	53
3	11.6164	443.818	77	1.17632	0.521444	9.00169	0.927711	0.77	2950	38.8312	40	34	46
4	14.4163	456.568	70	1.03228	0.525744	8.44031	0.846076	0.7	1970	56.7163	38	46	68
5	18.1947	45.1316	114	1.265	0.410744	12.0018	0.912	0.730769	1892	34.1404	35	27	41
6	21.18	172.59	100	1.52012	0.753154	11.2038	0.846076	0.714386	3840	38.1	39	31	47
7	25.1143	489.143	140	1.52652	0.753534	11.2012	0.903226	0.671077	4072	29.0857	30	27	35
8	35.8168	433.805	189	1.95343	0.859145	13.2012	0.903226	0.671077	4072	66.709	68	51	84
9	35.8447	150.616	116	1.27031	0.414445	12.1519	0.919355	0.710769	4004	35.1278	37	28	41
10	21.688	113.541	125	1.44331	0.721049	12.1517	0.905787	0.61414	7138	57.104	40	44	59
11	21.9072	349.684	133	1.23456	0.54442	11.0131	0.917241	0.710769	4004	31.6391	32	25	39
12	28.4991	68.4407	113	1.33116	0.660445	11.5918	0.911328	0.721359	4075	36.0619	37	29	46
13	30.1857	380.439	196	1.80157	0.831803	13.7973	0.903226	0.604811	5557	28.4176	29	23	34
14	34.7162	211.455	143	1.07849	0.374693	13.4935	0.923561	0.795214	6053	45.1452	45	34	58
15	40.0118	37.619	232	1.38806	0.691066	17.9125	0.846228	0.732717	11427	36.2131	39	30	46
16	45.7623	123.623	122	1.10508	0.435606	12.4634	0.917293	0.792051	4442	20.951	32	25	37
17	48.1936	203.731	134	1.40139	0.781897	17.0619	0.917043	0.671077	4004	41.5041	42	32	52
18	50.717	68.5566	212	1.49814	0.744424	16.4294	0.917043	0.671077	4004	39.5761	39	31	49
19	51.1043	461.739	92	1.44718	0.712855	16.4292	0.917043	0.671077	4004	31.6391	32	25	39
20	46.0732	162.192	478	1.03578	0.260563	24.67	0.917043	0.671077	4004	31.6391	32	25	39
21	44.6373	121.408	120	1.21481	0.567782	17.7468	0.923561	0.795214	6053	31.6391	32	25	39
22	48.3801	270.181	221	1.59845	0.780155	16.7144	0.923561	0.795214	6053	31.6391	32	25	39
23	44.7356	335.558	156	1.34108	0.743371	14.0935	0.923561	0.795214	6053	31.6391	32	25	39
24	44.7917	50.0764	144	2.34108	0.693726	13.5408	0.923561	0.795214	6053	31.6391	32	25	39
25	47.2033	473	93	1.33133	0.662074	16.8817	0.903226	0.671077	4004	31.6391	32	25	39
26	72.4838	281.915	117	1.44704	0.720491	12.7053	0.903226	0.671077	4004	31.6391	32	25	39
27	72.382	401.976	189	2.01167	0.807653	13.5128	0.903226	0.671077	4004	31.6391	32	25	39
28	76.4714	134.731	134	1.70364	0.809653	13.0419	0.903226	0.671077	4004	31.6391	32	25	39
29	74.6436	109.149	107	1.23687	0.590137	11.672	0.903226	0.671077	4004	31.6391	32	25	39
30	85.2195	188.102	147	2.37681	0.904641	13.4809	0.903226	0.671077	4004	31.6391	32	25	39
31	84.7927	74.7411	103	1.16561	0.511778	11.9918	0.903226	0.671077	4004	31.6391	32	25	39
32	94.4098	208.4	103	1.49479	0.711048	21.6321	0.903226	0.671077	4004	31.6391	32	25	39
33	90.5586	140.101	109	1.57451	0.717416	11.7806	0.903226	0.671077	4004	31.6391	32	25	39
34	94.5458	443.579	273	1.91649	0.833109	18.6639	0.903226	0.671077	4004	31.6391	32	25	39
35	98.7327	247.333	299	2.42403	0.748176	19.5115	0.903226	0.671077	4004	31.6391	32	25	39
36	98.7214	166.452	91	1.67096	0.601155	10.8817	0.903226	0.671077	4004	31.6391	32	25	39
37	104.712	441.98	150	2.13662	0.803736	16.1556	0.903226	0.671077	4004	31.6391	32	25	39
38	112.472	222.761	117	1.95261	0.784117	13.8188	0.903226	0.671077	4004	31.6391	32	25	39
39	111.844	344.631	149	1.39004	0.698828	13.7736	0.903226	0.671077	4004	31.6391	32	25	39
40	110.851	431.558	106	1.31794	0.65137	11.4174	0.903226	0.671077	4004	31.6391	32	25	39
41	120.828	192.143	84	1.15403	0.50172	10.3418	0.903226	0.671077	4004	31.6391	32	25	39
42	137.15	654.345	232	2.49597	0.649596	17.9125	0.903226	0.671077	4004	31.6391	32	25	39
43	141.443	128.974	305	2.04372	0.874693	19.7043	0.903226	0.671077	4004	31.6391	32	25	39
44	141.141	215.94	318	1.50812	0.748532	20.1535	0.903226	0.671077	4004	31.6391	32	25	39
45	141.141	215.94	318	1.50812	0.748532	20.1535	0.903226	0.671077	4004	31.6391	32	25	39
46	141.141	215.94	318	1.50812	0.748532	20.1535	0.903226	0.671077	4004	31.6391	32	25	39
47	141.141	215.94	318	1.50812	0.748532	20.1535	0.903226	0.671077	4004	31.6391	32	25	39
48	145.357	17.4886	207	1.77589	0.461311	12.1097	0.903226	0.671077	4004	31.6391	32	25	39
49	153.278	412.183	115	1.65246	0.461311	12.1097	0.903226	0.671077	4004	31.6391	32	25	39
50	159.683	278.456	134	2.00944	0.746105	12.1097	0.903226	0.671077	4004	31.6391	32	25	39
51	159.706	91.2227	243	2.00944	0.746105	12.1097	0.903226	0.671077	4004	31.6391	32	25	39
52	159.59	591.024	87	1.4046	0.746105	12.1097	0.903226	0.671077	4004	31.6391	32	25	39
53	164.983	500.34	315	1.69183	0.406417	14.9271	0.903226	0.671077	4004	31.6391	32	25	39
54	170.653	257.131	315	1.34436	0.649375	21.651	0.903226	0.671077	4004	31.6391	32	25	39
55	177.162	54.2575	147	1.20734	0.540216	10.9581	0.903226	0.671077	4004	31.6391	32	25	39
56	178.5	387.358	95	2.7007	0.918922	16.8001	0.903226	0.671077	4004	31.6391	32	25	39
57	182.152	125.777	224	1.22747	0.584033	11.8945	0.903226	0.671077	4004	31.6391	32	25	39
58	179.127	459.119	101	1.22747	0.584033	11.8945	0.903226	0.671077	4004	31.6391	32	25	39
59	173.359	190.781	128	1.64455	0.440433	11.8945	0.903226	0.671077	4004	31.6391	32	25	39
60	145.464	303.145	110	1.64455	0.440433	11.8945	0.903226	0.671077	4004	31.6391	32	25	39
61	197.076	139.223	223	1.64455	0.440433	11.8945	0.903226	0.671077	4004	31.6391	32	25	39
62	194.681	214.072	138	2.31237	0.894251	13.2555	0.903226	0.671077	4004	31.6391	32	25	39
63	199.681	455.06	313	1.71704	0.811482	11.2918	0.903226	0.671077	4004	31.6391	32	25	39
64	207.119	282.204	226	1.91002	0.655302	16.5813	0.903226	0.671077	4004	31.6391	32	25	39
65	203.444	35.0173	117	1.24158	0.655302	16.5813	0.903226	0.671077	4004	31.6391	32	25	39
66	206.23	259.208	178	1.43902	0.480437	15.0515	0.903226	0.671077	4004	31.6391	32	25	39
67	210.406	15.8125	128	1.43902	0.480437	15.0515	0.903226	0.671077	4004	31.6391	32	25	39
68	220.184	43.6389	461	1.14472	0.472599	24.2273	0.903226	0.671077	4004	31.6391	32	25	39
69	225.178	214.767	163	1.14472	0.472599	24.2273	0.903226	0.671077	4004	31.6391	32	25	39
70	230.978	235.904	146	1.31912	0.65216	11.4343	0.903226	0.671077	4004	31.6391	32	25	39
71	234.413	455.272	346	2.33131	0.903226	20.9881	0.903226	0.671077	4004	31.6391	32	25	39
72	235.502	132.459	255	1.7444	0.820288	18.0188	0.903226	0.671077	4004	31.6391	32	25	39
73	231.124	391.719	87	1.45186	0.724578	16.4551	0.903226	0.671077	4004	31.6391	32	25	39
74	242.341	156.406	251	2.26019	0.894797	17.0769	0.903226	0.671077	4004	31.6391	32	25	39
75	242.341	189.286	142	1.74517	0.820584	14.3618	0.903226	0.671077	4004	31.6391	32	25	39
76	238.514	24.4551	138	1.77073	0.825268	13.2555	0.903226	0.671077	4004	31.6391	32	25	39

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1	1	21.2188	366.125	64	1.29161	0.611372	9.03703	0.473172	0.727273	3642	56.8063	57	44	56
2	1	24.0233	451.446	129	1.42866	0.612321	12.8158	0.721429	0.661538	3872	30.0155	30	23	37
3	1	33.4428	114.293	140	1.45761	0.72755	13.3512	0.700000	0.72755	4313	30.8071	31	23	38
4	1	38.9272	251.374	206	1.6079	0.79483	15.1953	0.818643	0.727432	5016	43.7573	45	35	53
5	1	38.5562	278.849	178	1.06005	0.731755	15.0543	0.927003	0.791111	9196	51.6579	52	38	64
6	1	39.3667	61.32	150	1.17668	0.571089	13.8198	0.720245	0.765306	4709	31.3933	33	25	38
7	1	38.3765	348.386	85	1.17668	0.527265	10.4031	0.494727	0.700333	3880	46.4235	69	39	53
8	1	45.2733	24.1727	139	1.40448	0.703172	13.1031	0.925237	0.847426	4278	30.7642	32	25	36
9	1	51.1316	103.948	161	1.77145	0.835428	15.4504	0.921188	0.618267	4110	23.9879	24	18	29
10	1	55.8904	67.5278	146	1.61559	0.745435	13.6143	0.927031	0.695238	4298	28.4344	30	22	36
11	1	55.5476	126.373	176	1.31687	0.600118	12.666	0.927031	0.695238	4298	32.5238	33	25	40
12	1	65.4146	170.866	374	2.47732	0.916453	20.3108	0.75807	0.482143	16200	50.7821	52	39	61
13	1	61.8915	292.118	195	1.45938	0.790015	15.757	0.919911	0.714246	8750	48.5	50	37	59
14	1	63.3444	214.032	180	1.7276	0.825678	15.1388	0.904573	0.714286	8750	74.8	74	56	91
15	1	72.2722	275.178	45	1.42838	0.716165	15.5694	0.918347	0.716286	5366	79.7286	74	56	91
16	1	74.2075	362.453	153	1.46508	0.730839	13.9573	0.9271687	0.733	4772	69.2019	69	50	80
17	1	83.9178	94.1466	192	1.14916	0.492694	6.21872	0.913793	0.716111	2514	64.2019	69	50	80
18	1	96.0609	317.476	115	1.27619	0.621756	15.6353	0.923077	0.716195	8905	51.4596	64	36	59
19	1	98.0948	190.518	197	1.14488	0.484908	15.8716	0.920543	0.724245	9210	55.8035	61	31	49
20	1	96.4796	167.951	103	1.17642	0.538986	11.4518	0.895652	0.72024	4104	32.0443	42	31	51
21	1	102.493	121.97	203	2.8612	0.938412	16.0769	0.835393	0.463364	9738	32.0443	42	31	51
22	1	100.951	38.6619	122	1.77624	0.813318	12.4631	0.917293	0.672449	3820	28.1819	30	27	35
23	1	101.027	99.8591	149	1.66847	0.841728	13.7736	0.923666	0.62222	6149	32.9308	35	30	46
24	1	99.8	243.069	130	1.27609	0.621728	12.8655	0.902778	0.716753	4134	35.9478	37	28	44
25	1	104.131	619.385	109	1.17019	0.519347	11.7806	0.915966	0.736944	4199	44.7599	47	33	51
26	1	104.156	54.1167	199	1.51352	0.642345	10.7047	0.929907	0.763395	8907	43.9722	45	33	51
27	1	104.156	111.911	90	1.22763	0.575349	12.1005	0.917298	0.716753	4134	35.9478	37	28	44
28	1	110.172	713.843	315	1.22763	0.575349	12.1005	0.920655	0.818182	4016	45.9504	51	47	58
29	1	110.172	98.9077	81	1.21993	0.57861	11.1704	0.895083	0.716728	3924	40.0408	41	33	58
30	1	116.382	401.511	91	1.59191	0.719849	15.9577	0.917421	0.712868	9236	46.18	47	33	51
31	1	116.382	59.645	200	1.28836	0.630508	11.2772	0.895652	0.686867	4065	42.0009	47	33	51
32	1	128.94	171.05	99	1.71763	0.813046	11.4518	0.923333	0.70814	9756	35.2702	37	28	44
33	1	128.94	449.753	103	1.75132	0.715144	16.78	0.90991	0.763152	4329	40.8872	42	33	46
34	1	130.316	196.913	277	1.19599	0.504271	11.3401	0.916667	0.667857	9071	46.508	50	37	65
35	1	130.316	628.588	101	1.49519	0.837336	15.4304	0.929825	0.781259	4167	35.3113	40	31	44
36	1	141.977	497	106	1.45728	0.72588	11.6174	0.929825	0.781259	4167	35.3113	40	31	44
37	1	141.977	321.56	84	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
38	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
39	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
40	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
41	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
42	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
43	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
44	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
45	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
46	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
47	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
48	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
49	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
50	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
51	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
52	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
53	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
54	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
55	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
56	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
57	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
58	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
59	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
60	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
61	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
62	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
63	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
64	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
65	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
66	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
67	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
68	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
69	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
70	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
71	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
72	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
73	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
74	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
75	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
76	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
77	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
78	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
79	1	141.977	478.113	106	1.39597	0.697244	10.3118	0.903226	0.777779	4132	48.9524	49	36	61
80	1	141.977	478.113	106	1.3									

1	9	213.465	101.811	127	1.4886	0.805787	12.7162	0.913669	0.461459	4625	36.4173	37	29	44
9	86	214.615	440.671	173	1.51036	0.739944	14.8435	0.925114	0.758772	7670	44.3153	46	35	59
10	91	215.665	289.917	94	1.51636	0.758311	10.3418	0.907316	0.661514	4211	50.3131	51	38	62
11	96	216.312	80.4481	154	1.51737	0.670464	14.0028	0.816457	0.773733	4524	29.1936	70	36	66
12	97	217.989	305.311	90	1.50948	0.753714	10.7047	0.900091	0.754802	4134	45.9333	46	34	56
13	98	219.374	47.9316	107	1.37271	0.685038	11.897	0.808811	0.754786	4037	18.0093	19	29	44
14	99	202.5	332.755	106	1.53544	0.758839	11.4714	0.913053	0.61377	4706	44.2194	41	36	51
15	90	310.581	348.784	148	2.38795	0.908085	13.7273	0.860909	0.519531	8932	60.4327	40	61	74
16	91	305.159	344.311	44	1.29933	0.637205	17.8882	0.846154	0.4875	3740	45.457	88	65	106
17	92	313.137	388.246	224	2.05865	0.874094	16.888	0.912184	0.59895	5246	41.2768	41	37	50
18	93	312.337	242.253	358	2.14387	0.881658	21.3793	0.825751	0.735641	19817	55.2004	56	47	69
19	94	315.082	332.118	182	1.63477	0.738934	15.2227	0.925531	0.636482	9474	27.0549	55	42	64
20	95	332.65	51.4736	143	1.82189	0.635902	14.4982	0.810615	0.639216	4361	26.7171	38	20	37
21	96	332.5	256.405	158	1.26018	0.624816	14.4582	0.923977	0.552781	4988	31.5496	33	25	38
22	97	337.5	45.3644	251	1.64039	0.679838	17.5172	0.923977	0.634211	9916	41.1152	42	31	51
23	98	342.921	422.568	241	1.73019	0.661977	13.8658	0.895911	0.634211	9916	41.1152	42	31	51
24	99	344.143	139.601	28	1.21949	0.661977	13.8658	0.920732	0.725962	4244	28.1191	29	27	41
25	100	352.044	292.616	173	1.60646	0.660466	5.97082	0.875	0.666667	3659	202.107	215	166	210
26	9	358.063	396.431	145	2.26617	0.936313	14.8435	0.935135	0.758772	6899	27.1418	28	21	33
27	101	348.12	70.9816	217	2.76918	0.893818	16.4721	0.929487	0.742559	8423	38.0997	40	47	69
28	102	358.404	69.409	74	1.22379	0.637632	9.43498	0.844118	0.648983	4593	31.7551	21	17	26
29	103	358.161	37.455	11	1.18766	0.783935	3.74241	0.785314	0.6873	210	19.0026	99	14	22
30	104	358.261	202.516	168	2.20147	0.89901	14.5381	0.922232	0.65977	4586	27.6867	29	21	31
31	105	370.203	302.574	166	2.20147	0.950498	22.4058	0.930876	0.704794	20716	51.2732	52	37	46
32	106	372.012	170.053	404	1.31694	0.640136	10.4758	0.931818	0.788442	12022	36.6324	37	27	46
33	107	384.168	282.818	328	1.6475	0.784218	20.4318	0.891604	0.627273	5544	31.3123	52	41	62
34	108	375.438	482.112	44	1.5224									

EV Table 2.doc

Example of the summary output of AnalyseDNA.m program
(summary for 10 3 by 3 montage images)

1	1187	143.912	79.3918	1.59219	0.388735	0.726461	0.133996	14.0412	3.315	0.805327	0.0350165	0.701218	0.075176	6149.26	1456.35	61.539	18.352	62.9414	18.9393
2	1205	14.311	50.5906	22.6594	0.389942	0.727835	0.134442	14.2434	3.43288	0.906571	0.0311453	0.70177	0.0720289	6786.37	3095.1	62.2416	17.0965	63.0245	17.383
3	1265	169.016	86.8722	1.60511	0.397935	0.72552	0.13163	14.0453	3.33904	0.903269	0.0316822	0.702891	0.0720005	6881.04	3225.41	64.3167	20.5918	66.3818	21.2287
4	1388	16.2067	84.4117	25.1765	0.389682	0.727142	0.142518	14.3531	3.43812	0.902766	0.0374254	0.695845	0.0753889	6997.68	4212.87	63.1398	19.8382	64.9561	20.5112
5	1448	171.021	89.7145	1.60189	0.400493	0.721084	0.141204	14.338	3.45045	0.901152	0.0379801	0.70023	0.0756884	7050.22	4162.04	64.8559	21.6761	65.8522	22.1282
6	1419	165.112	84.4806	1.60542	0.425512	0.728414	0.133721	14.0974	3.38686	0.904814	0.0362731	0.696204	0.0782455	6843.2	3924.12	64.266	19.3396	65.9105	19.9724
7	1756	128.864	98.2039	1.51806	0.451022	0.694813	0.182667	11.726	5.27411	0.893311	0.0481729	0.704526	0.0892392	5162.51	4393.13	34.9743	21.1969	36.16	22.0024
8	1260	171.387	84.1254	1.59201	0.405167	0.723421	0.137559	14.3833	3.40593	0.906384	0.0357179	0.703574	0.0751602	6865.87	3767.48	62.2752	17.2223	63.9781	17.7621
9	1270	166.53	84.5094	1.60763	0.404986	0.72568	0.138548	14.1402	3.47794	0.905208	0.0366298	0.700331	0.0766773	6576.34	4022.38	61.6064	17.8994	63.163	18.2498
10	1425	159.106	82.618	1.53184	0.400372	0.717147	0.139065	13.8285	3.49536	0.904275	0.0399111	0.702759	0.07572	6507.18	3768.39	64.2141	20.7694	65.8765	21.135
	36.4582	16.3199	51.7958	23.6094															

CLAIMS

What is claimed is:

1. A method of predicting a property of a manipulation of cells based
5 upon a descriptor, said method comprising:
 providing a plurality of cells;
 manipulating said plurality of cells;
 capturing a morphological value from said plurality of cells;
 assigning a degree of presence of said morphological value; and
10 storing said morphological value and said degree of presence;
 wherein said descriptor is derived from a first component of a cell and
a second component of said cell, said capturing said morphometric value from said
plurality of cells comprises determining a relationship between said first component
and said second component.
- 15 2. The method of claim 1 wherein said first component and said second
component are selected from a protein, a protein modification, a nucleic acid, a lipid,
a carbohydrate, a subcellular structure and an organelle.
3. The method of 1 wherein said step of manipulation occurs in a manner
selected from a electrical source, a chemical source, a thermal source, a gravitational
20 source, a nuclear source, a temporal source, and a biological source
4. The method of claim 3 wherein said chemical source is selected from a
pharmacological candidate and a drug screening library.
5. The method of claim 1 wherein said morphological value is selected
from a count, an area, a perimeter, a length, a breadth, a fiber length, a fiber breadth, a
25 shape factor, a elliptical form factor, an inner radius, an outer radius, a mean radius,
an equivalent radius, an equivalent sphere volume, an equivalent prolate volume, an
equivalent oblate volume, an equivalent sphere surface area, an average gray value, a
total gray value, and an optical density.
6. The method of claim 1 wherein said degree of presence is
30 multiple of a quantized value.

7. A computer program product for populating a database with manipulated biological information, said computer program product comprising:
- code for providing a plurality of cells in various stages of the cell cycle, said stages of the cell cycle including at least one selected from interphase, G0 phase, G1 phase, S phase, G2 phase, M phase, prophase, prometaphase, metaphase, anaphase, and telophase;
 - code for manipulating said cells in said various stages of cell cycle development to form a plurality of manipulated cells;
 - code for capturing an image of said plurality of manipulated cells;
 - code for determining a descriptor from said image for said manipulated cells;
 - code for populating a database with said descriptor;
 - wherein said image includes a first component of a cell and a second component of said cell; and
 - a computer readable storage medium for holding the codes.
8. The computer program product of claim 7 wherein said first component and said second component are selected from a protein, a protein modification, a nucleic acid, a lipid, a carbohydrate, a sub-cellular structure and an organelle.
9. The computer program product of claim 7 wherein said image is a digitized representation of said plurality of manipulated cells.
11. The computer program product of claim 9 wherein said digitized representation provides a density value of said plurality of manipulated cells.
11. The computer program product of claim 7 wherein said descriptors comprise numeric or logical values.
12. The computer program product of claim 11 wherein said values further comprises a nucleotide.
13. The computer program product of claim 11 wherein said values further comprises an amino acid letter.
14. A system for capturing images of cells or cell structures, the system comprising:
- a cell holder comprising a plurality of sites in a spatial orientation, each of the sites being capable of holding a plurality of cells to be imaged;

an image capturing device coupled to the cell holder, the image capture device being adapted to capture at least one image in at least one of the plurality of sites;

an illumination apparatus comprising a liquid light guide coupled to the plate for highlighting the plurality of cells in a relatively even spatial manner for image capturing purposes;

an image processing device coupled to the image capturing device, the image capturing device being adapted to convert the image into a digital representation; and

a database storage device comprising a database management element coupled to the image capturing device, the database storage device being adapted to retrieve the digital representation of the image from the image processing device and storing the digital representation.

15. The system of claim 14 further comprising a stage comprising a device for moving the cell holder in a spatial direction to traverse across the cell holder in the spatial orientation.

16. The system of claim 14 wherein the illumination apparatus comprises sub-elements, at least one of the sub-elements being positioned away from the image capturing device to prevent a possibility of vibration from the one sub-elements to be transmitted to the image capturing device.

17. The system of claim 14 wherein the digital representation comprises a plurality of regions and objects.

18. The system of claim 14 further comprising a computing device connected between the database storage device and the image processing device.

19. The system of claim 14 wherein the image capturing device comprises a magnification of at least 1X and greater to capture the image of the site.

20. The system of claim 14 wherein the plurality of sites comprises at least 96 sites.

21. The system of claim 14 wherein the liquid light guide characterized as a flexible member that substantially prevents vibration from the an element of the illumination apparatus to be transferred to the image capturing device.

22. The system of claim 14 wherein the spatial direction can be selected from an x-direction, a y-direction, or a z-direction in a Cartesian coordinate system.

23. The system of claim 14 wherein the each of the sites comprises
5 a volume that is sufficient to prevent a solution therein from evaporating in a substantial manner that may influence the image capturing.

24. A method for identifying a mechanism of action for a first compound, the method comprising the steps of:
receiving the first compound;
10 measuring at least one feature of a cellular phenotype to define a target phenotype;
identifying additional compounds providing a feature similar to the feature identified in the measuring step; and
characterizing the first compound in terms of distance from a specific
15 target phenotype having known characteristics.

25. The method of claim 24 comprising the further step of storing the additional compounds and their associated phenotypes in a database.

26. A method for identifying a mechanism of action for a cellular stimulus, the method comprising the steps of:
20 receiving cells of interest;
measuring at least one feature of the cells to define and quantify a target phenotype;
identifying additional compounds providing a feature similar to the feature identified in the measuring step; and
25 characterizing the first compound in terms of distance from a specific target phenotype having known characteristics.

27. A method for identifying information relevant to at least one of a mechanism of action and cellular activity by utilizing assay data to elucidate a phenotype, the method comprising the steps of:
30 identifying a target protein;
identifying positive and negative biochemical hits related to the target protein;
defining the target phenotype utilizing the positive and negative hits;

identifying other compounds providing similar features; and
characterizing the first compound in terms of distance from a specific
target phenotype having known characteristics.

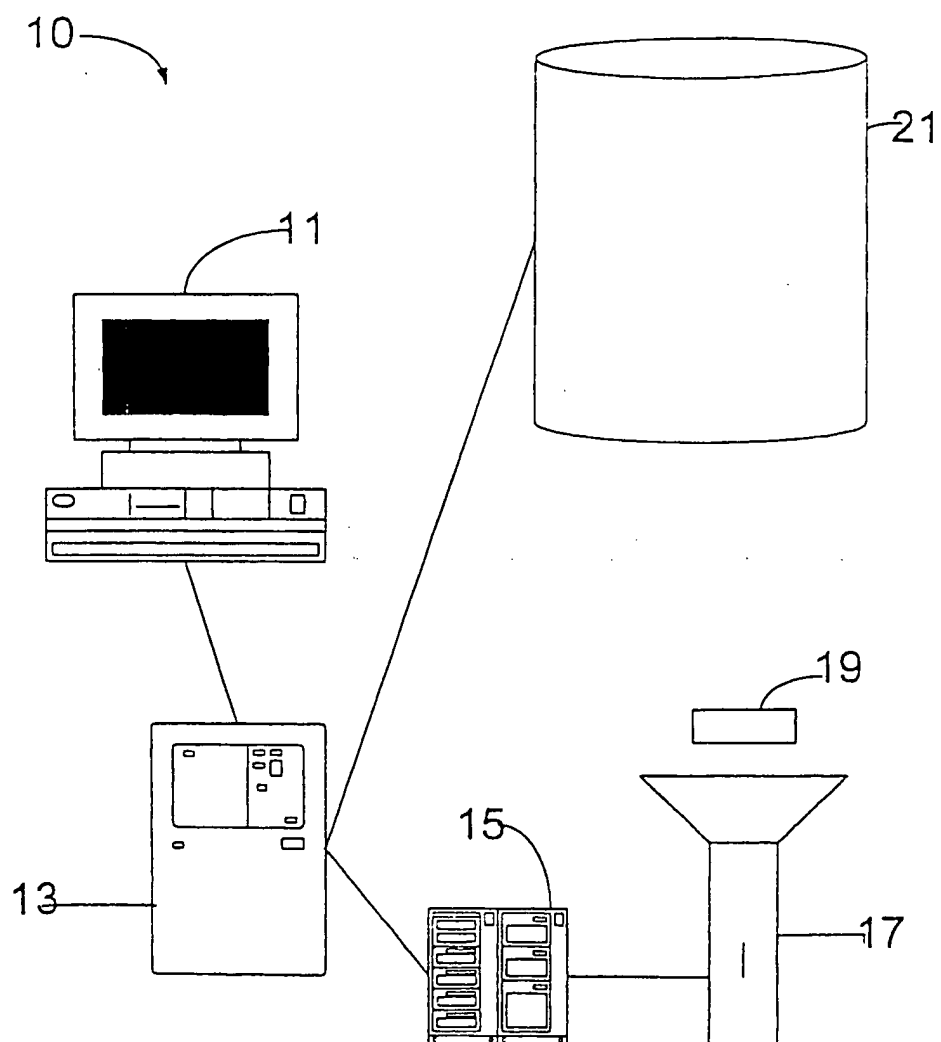


FIG. 1

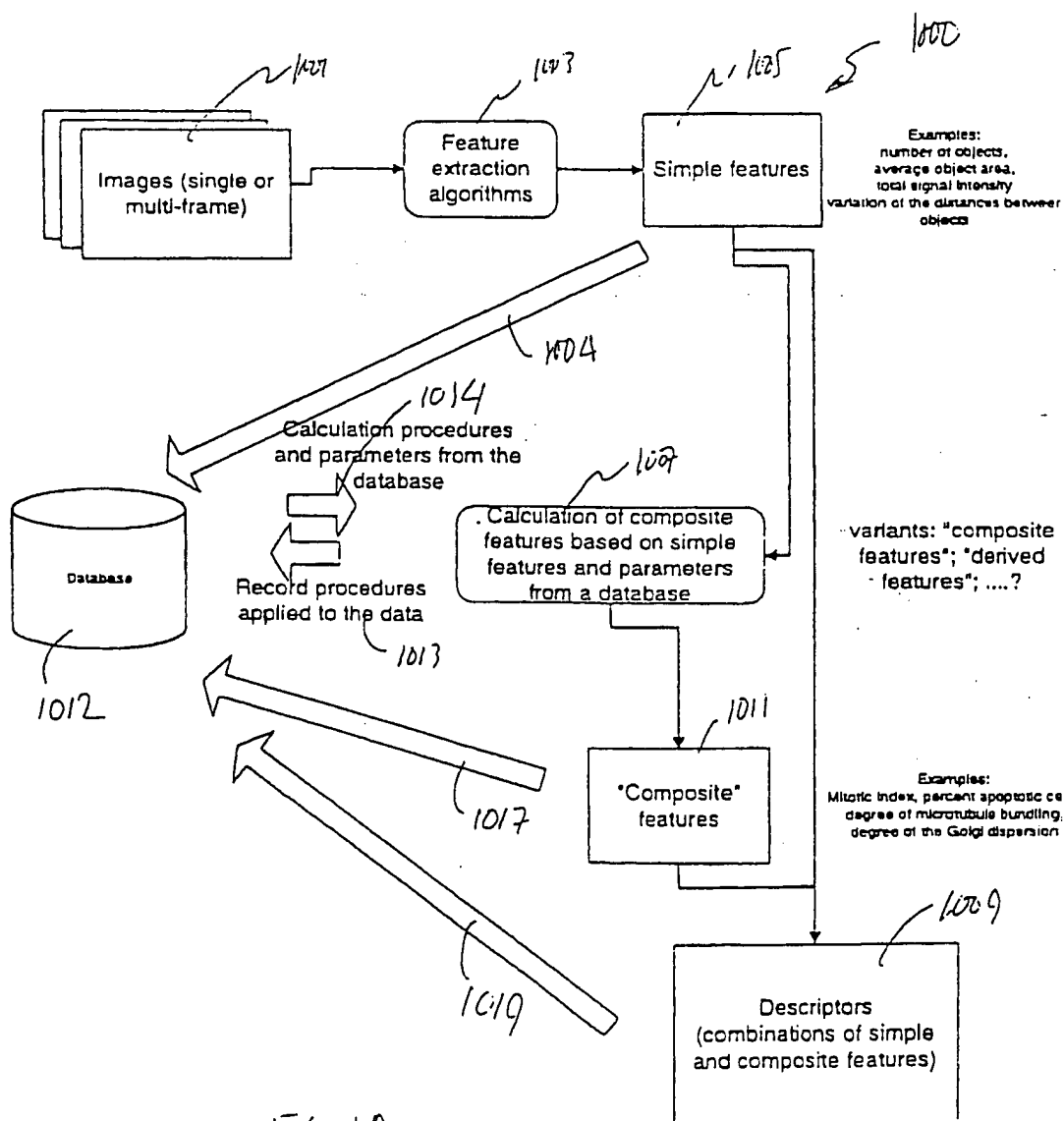


FIG. 1A

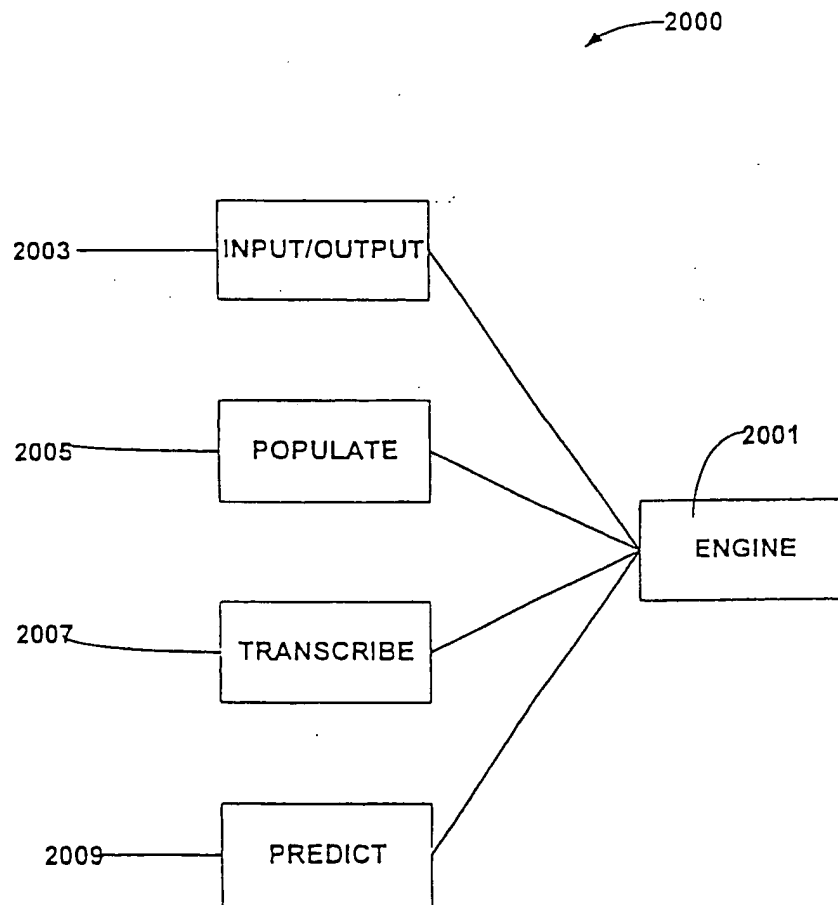


FIG. 1B

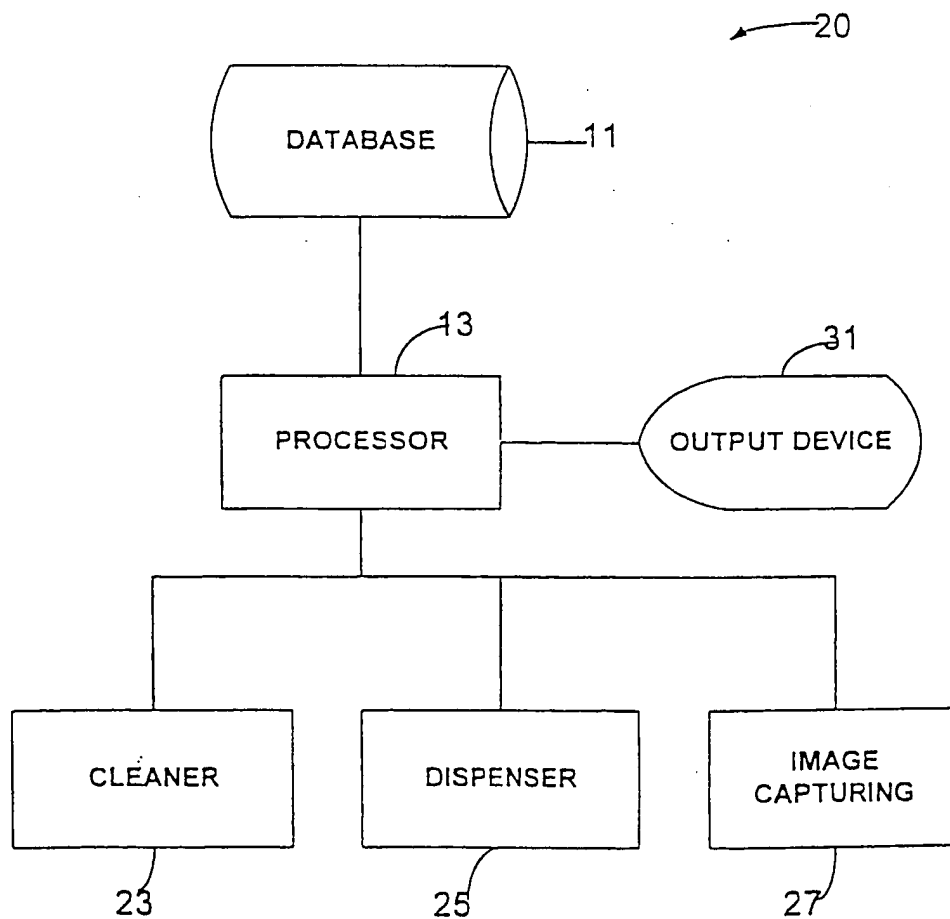


FIG. 2

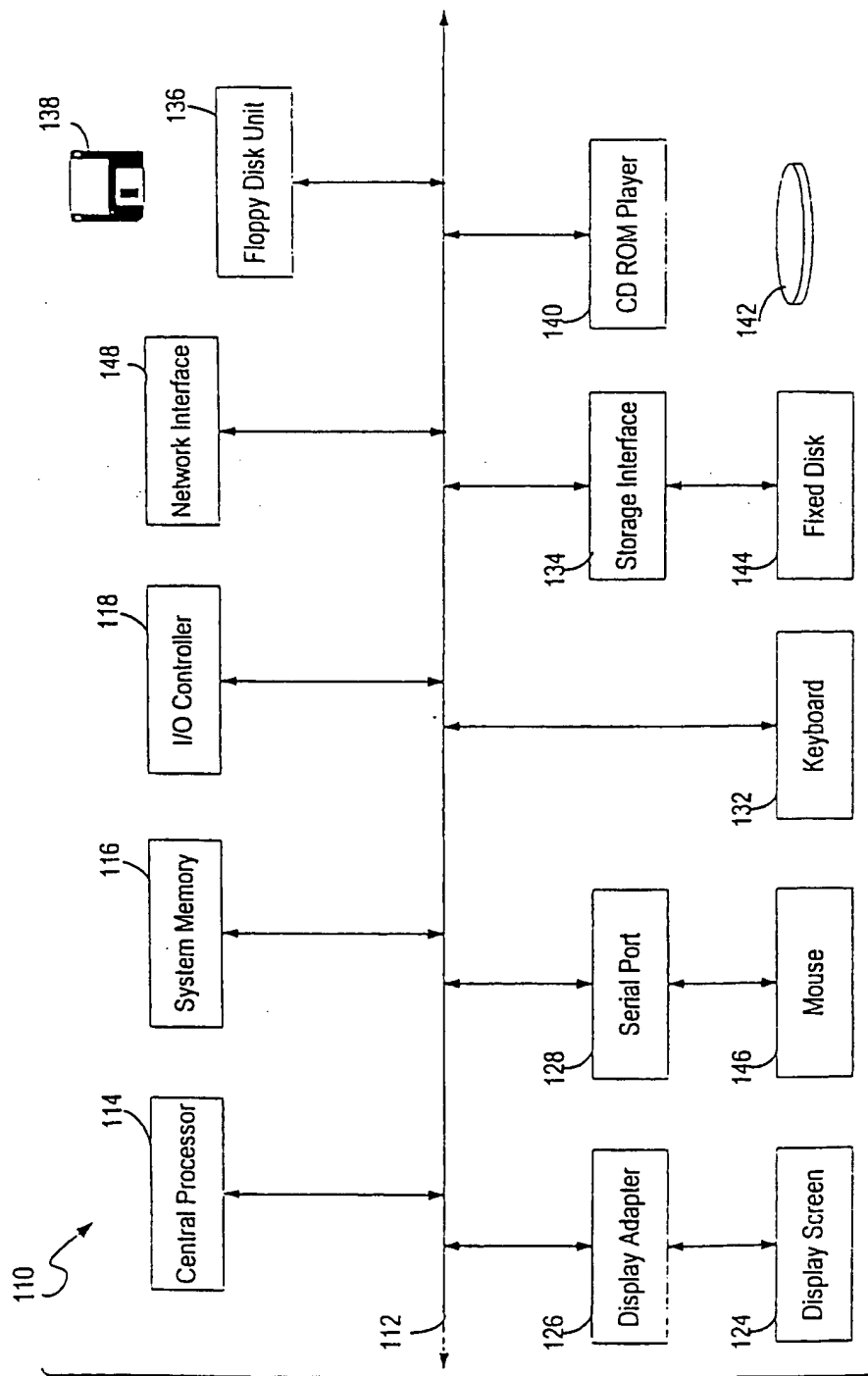


Fig 3

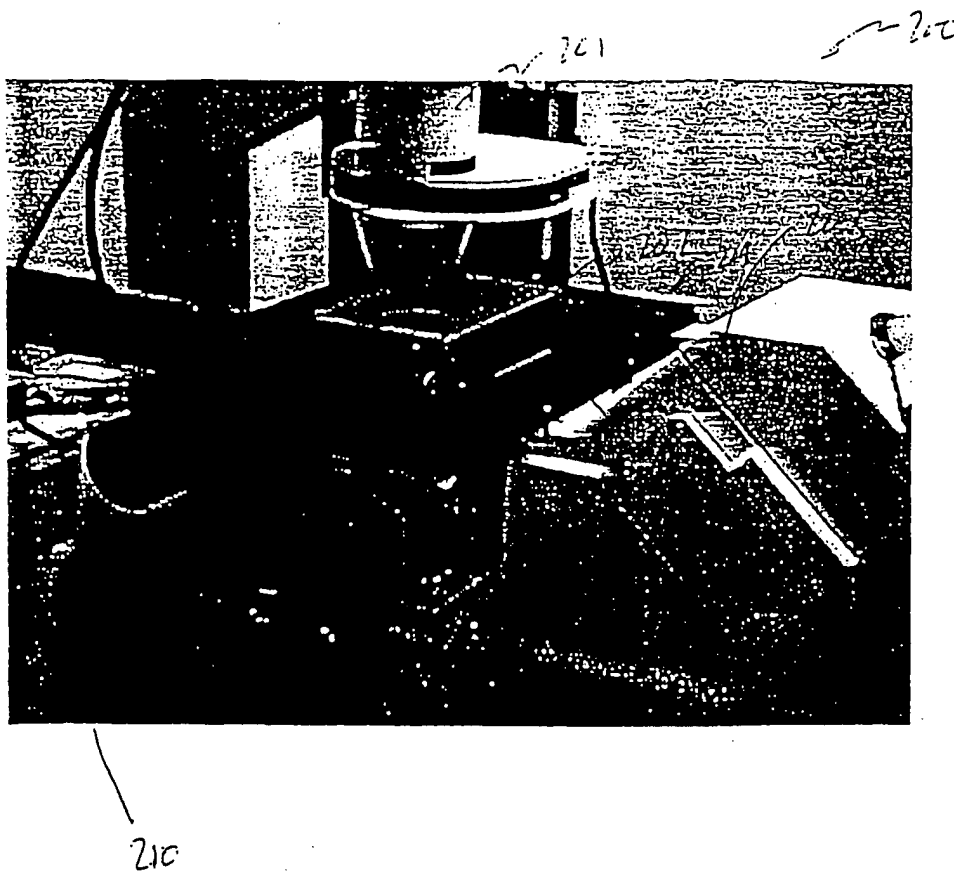


FIG. 4

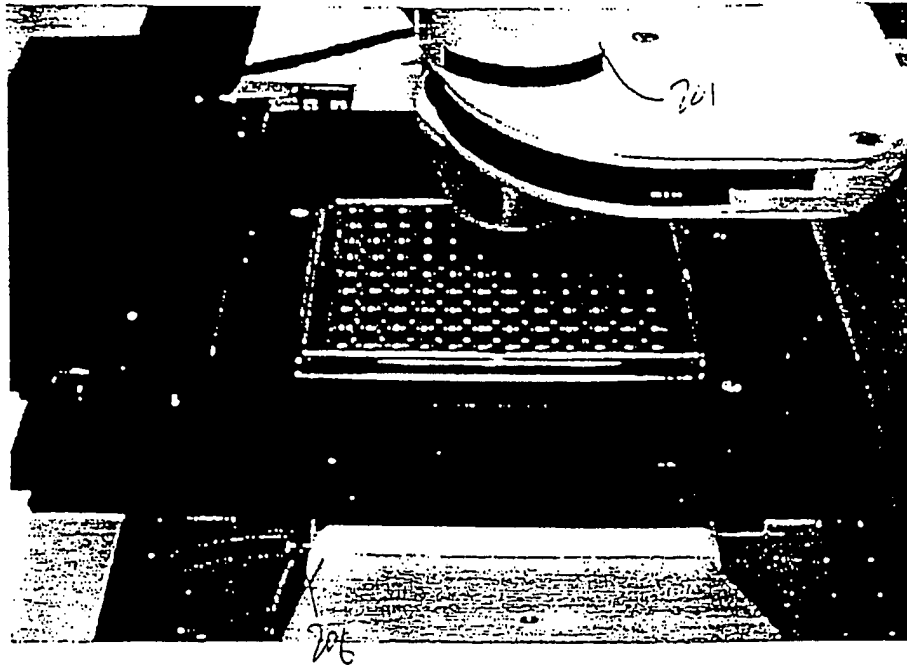
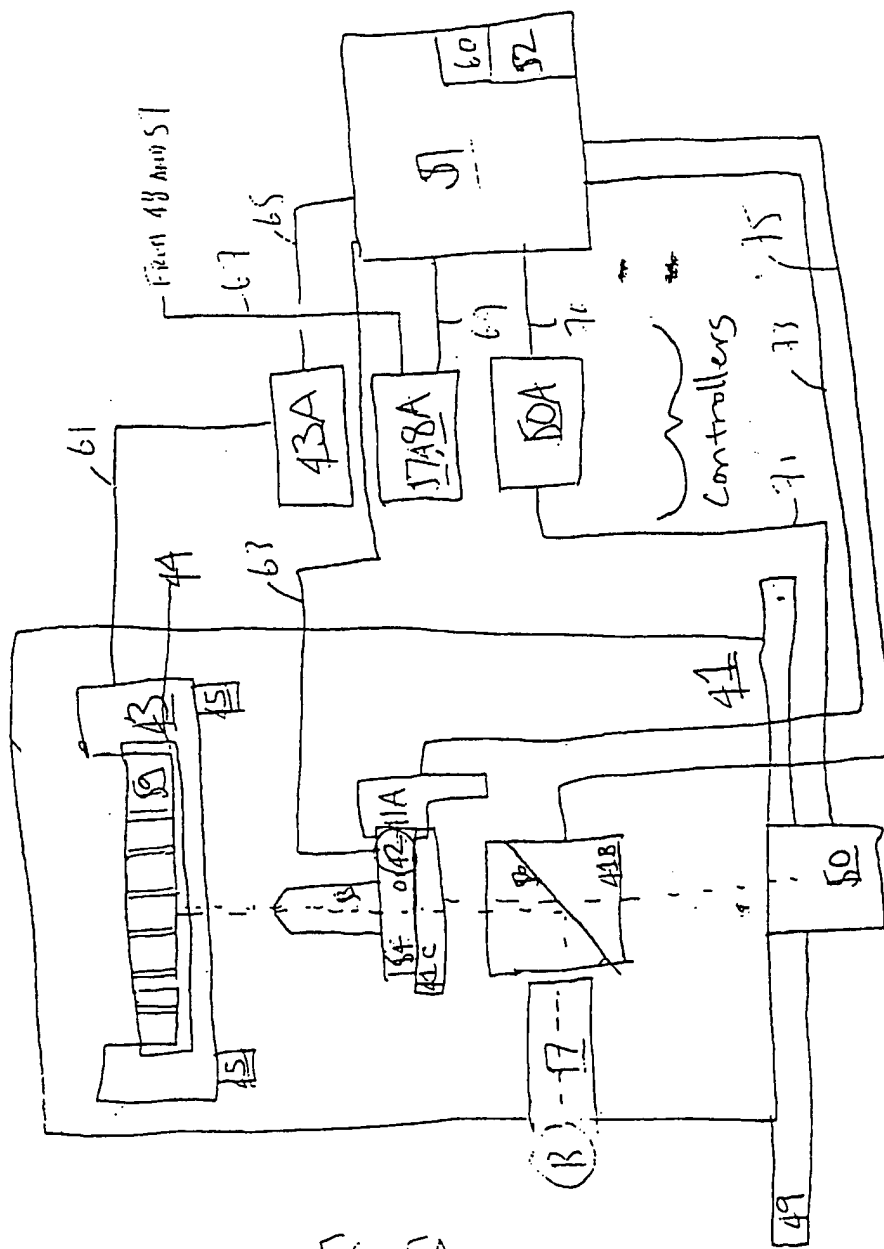


FIG 5



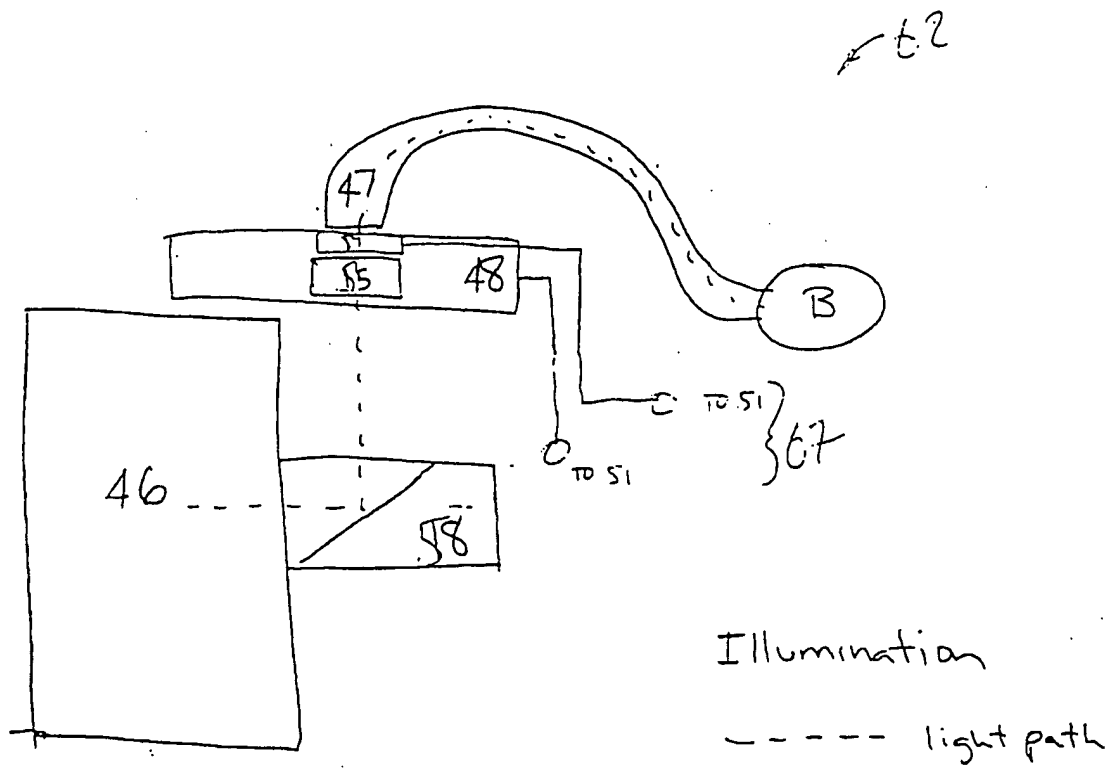


FIG. 5B

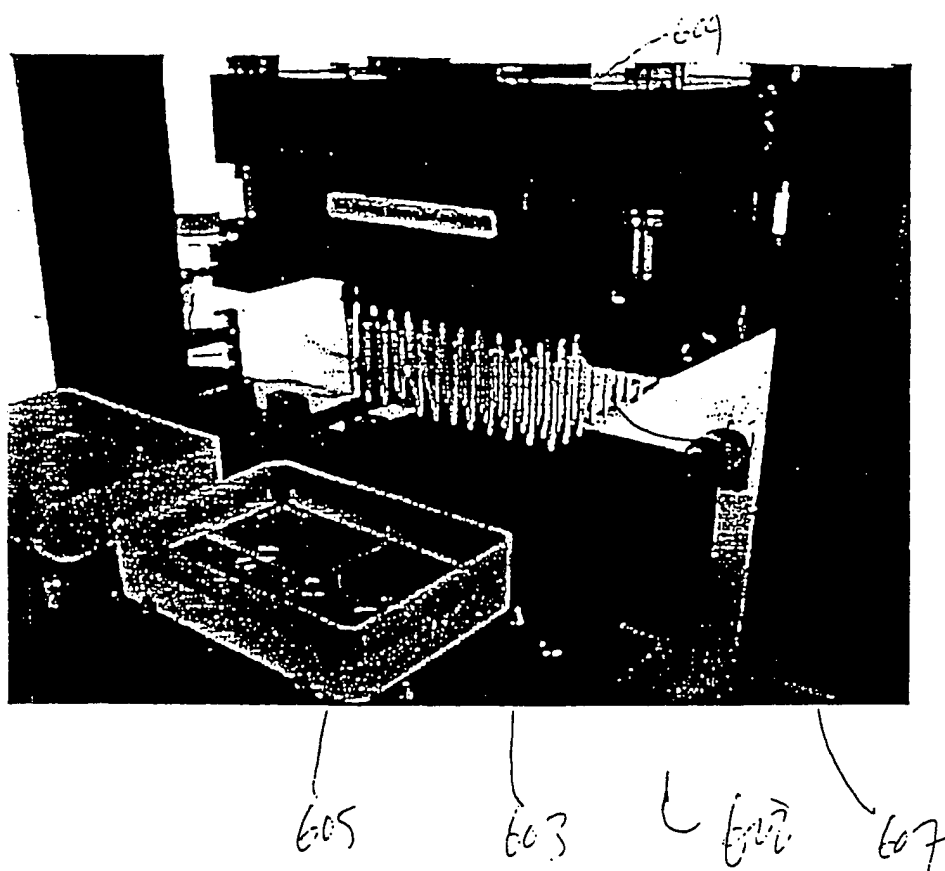


FIG. 6

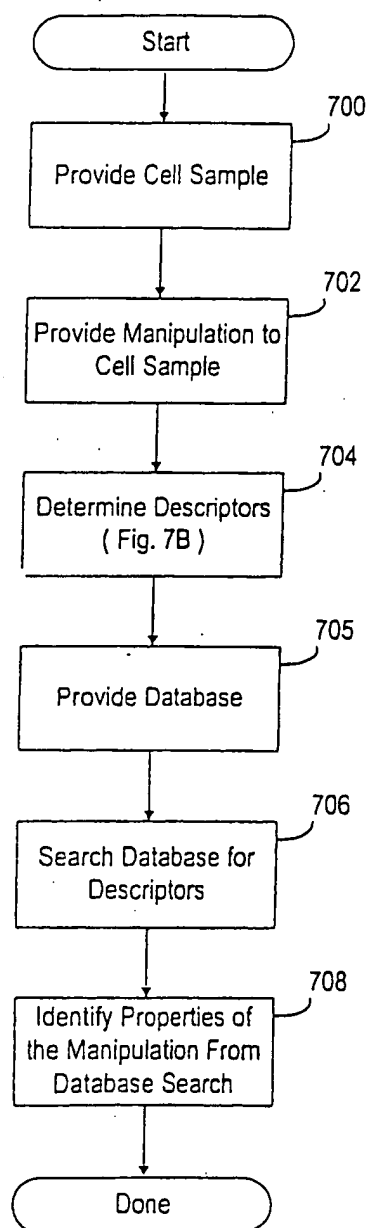
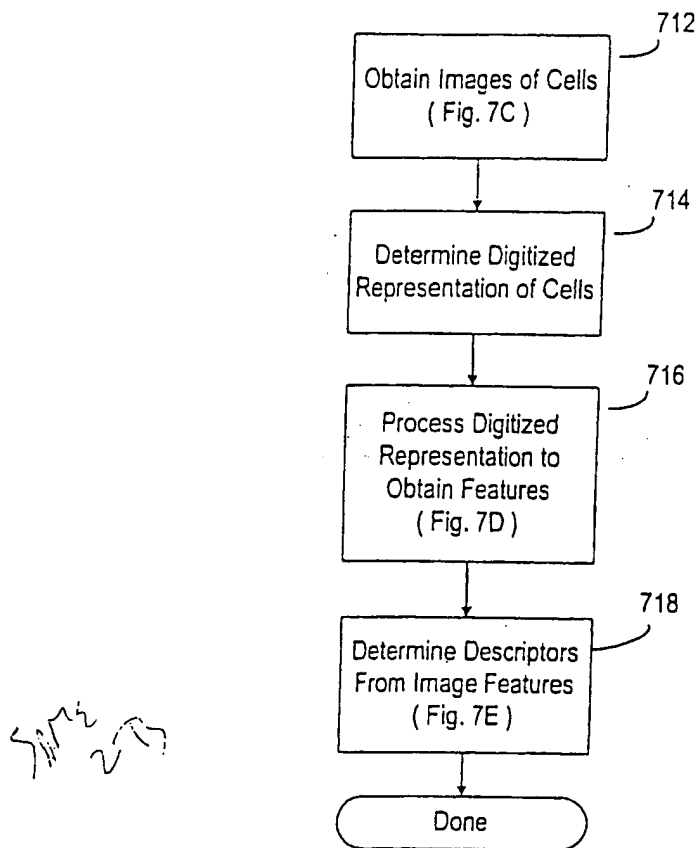


Fig. 7A



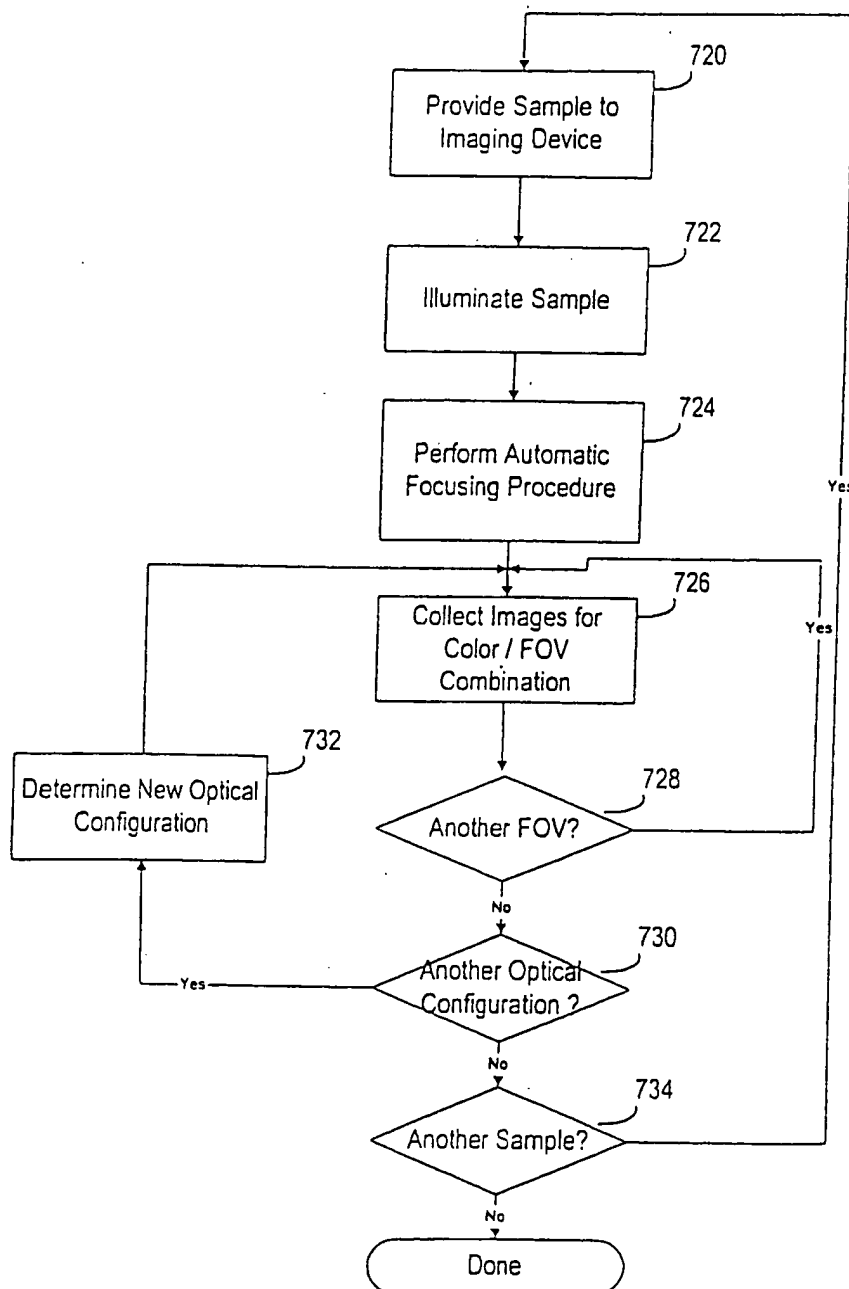


Fig. 7C
Step 714 of Fig. 7B

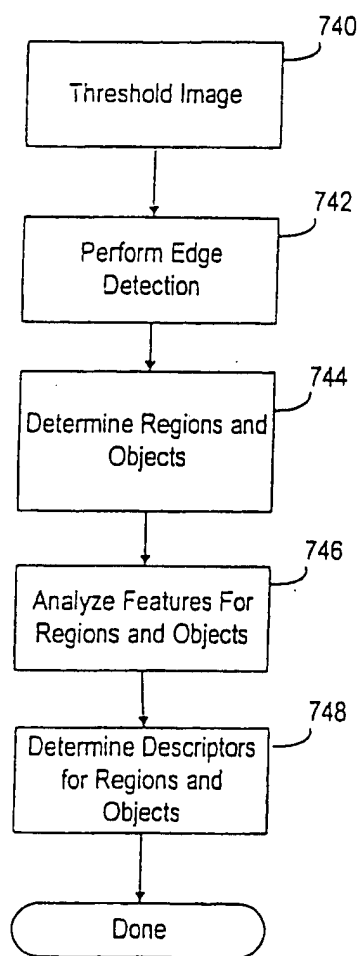


Fig. 7D
Step 716 of Fig. 7B

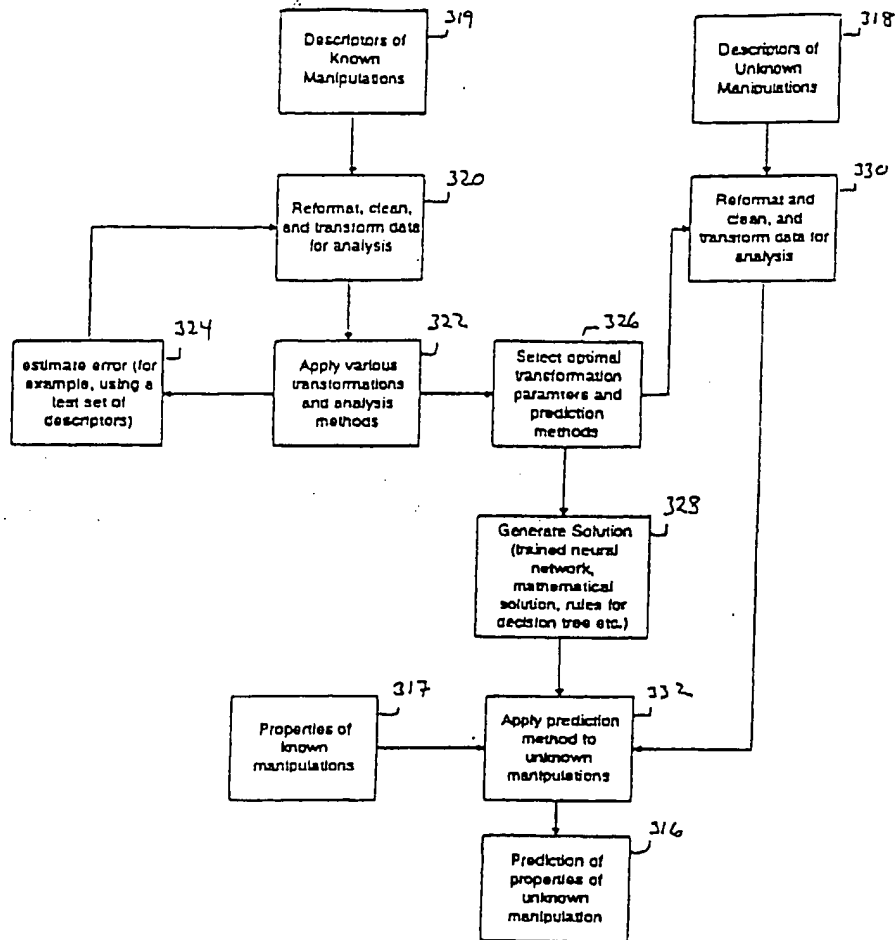


FIG. 7E

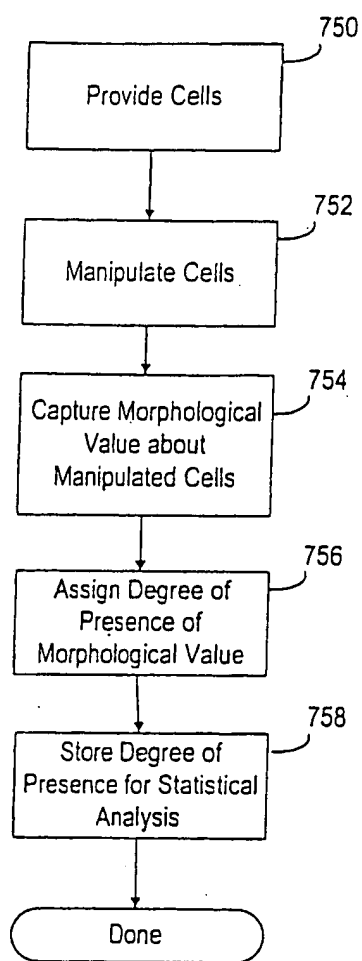


Fig. 7F

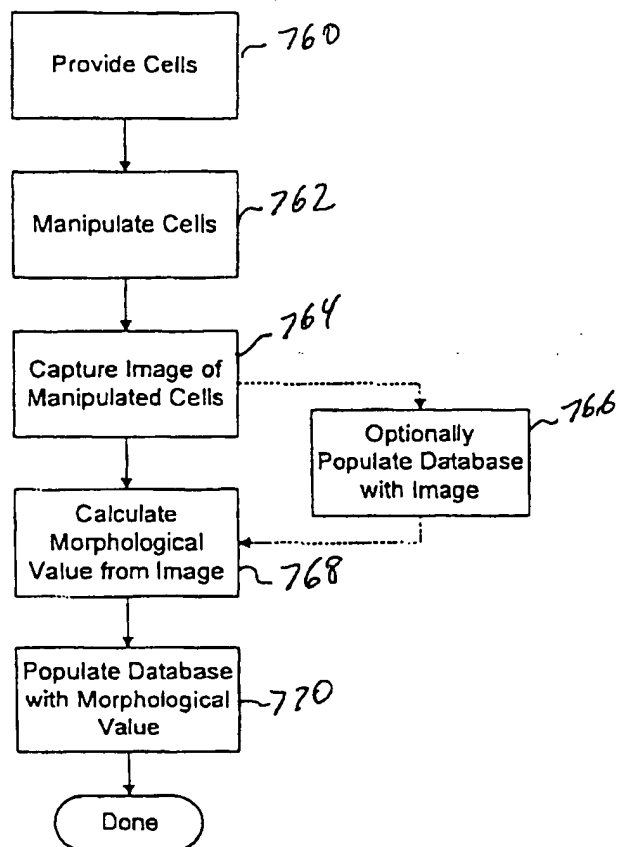


Fig 7G

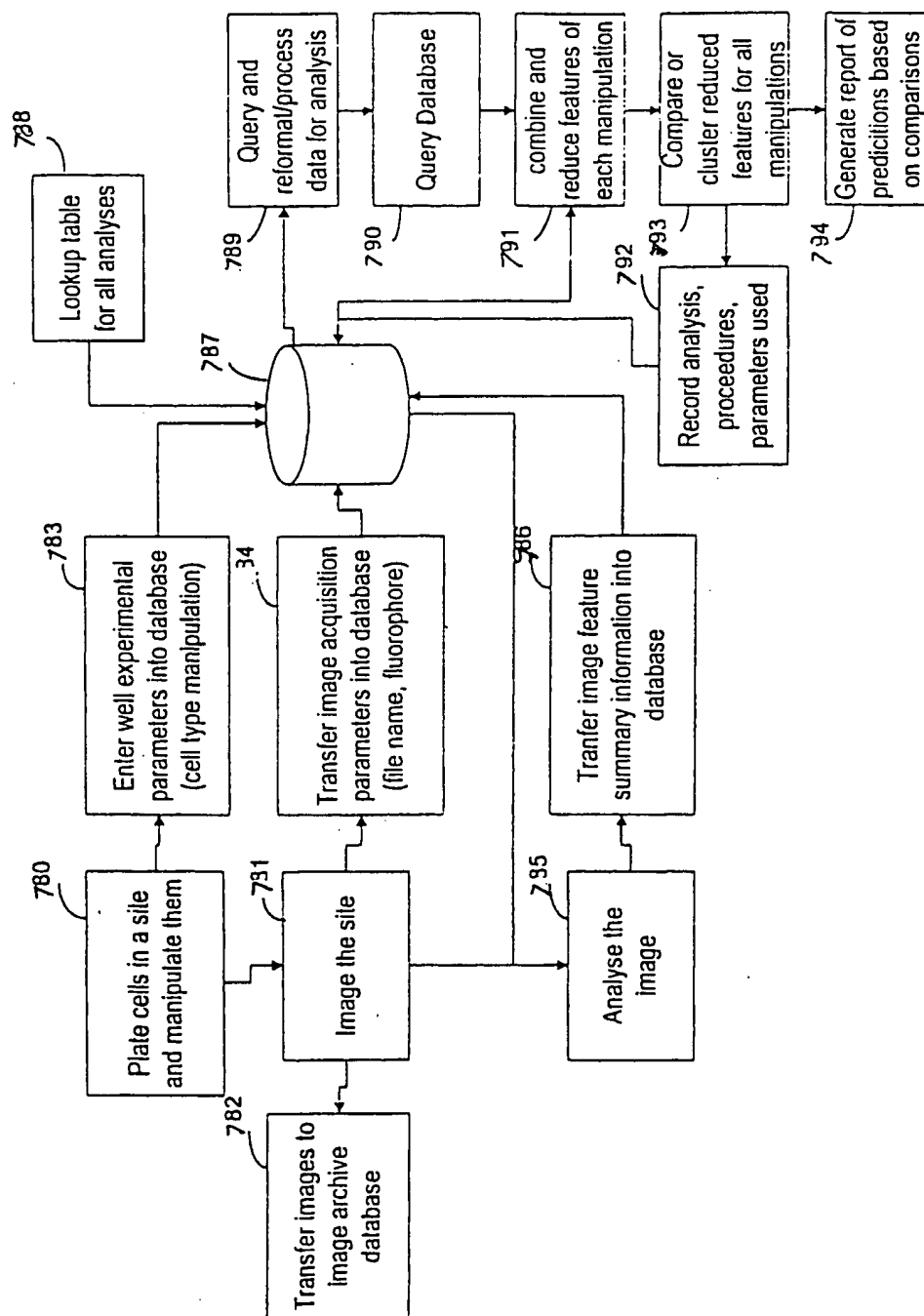


Fig.7 H

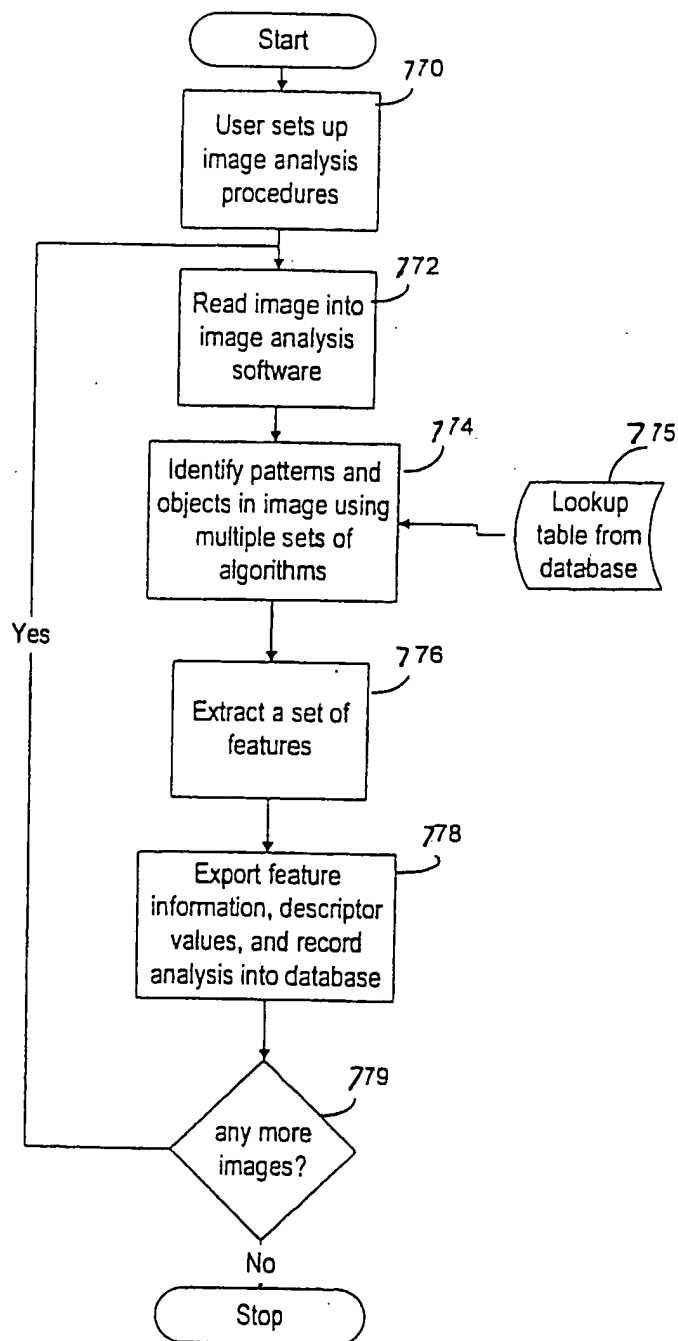


Fig. 71

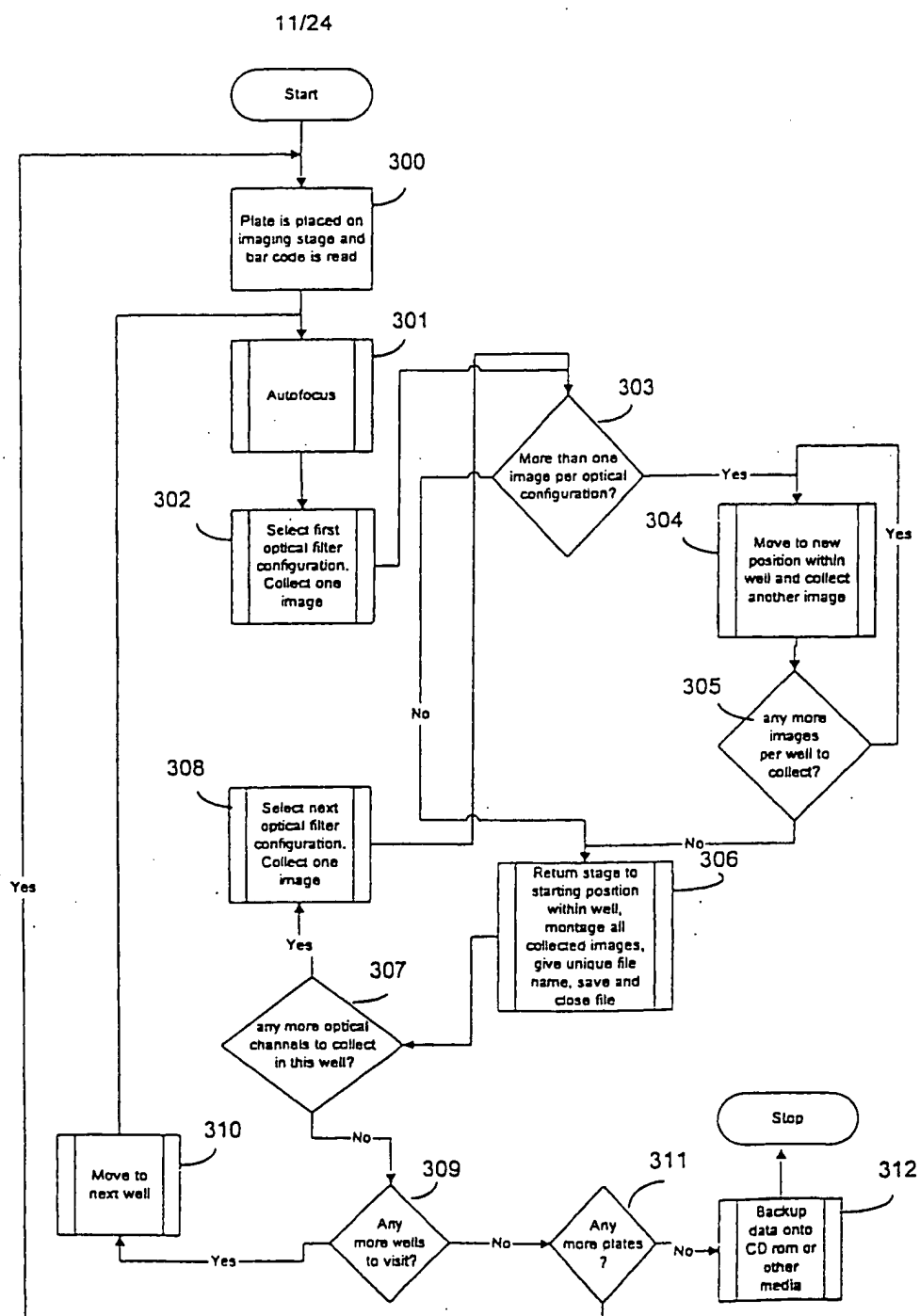


Fig. 7J

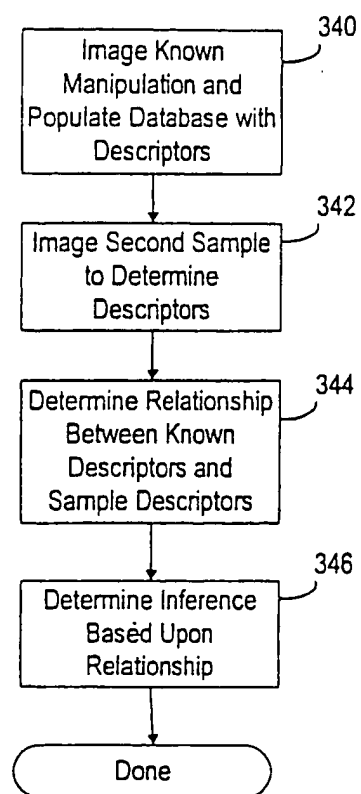


Fig. 7K

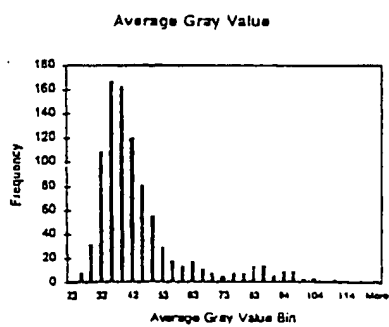


Fig. 8A

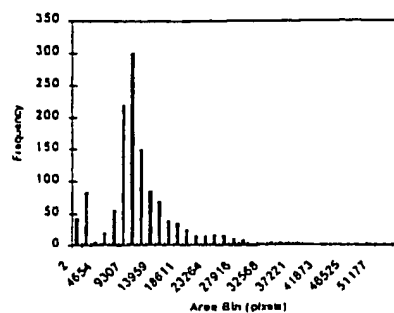


Fig. 8B

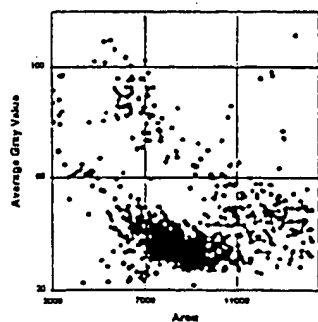


Fig. 8C

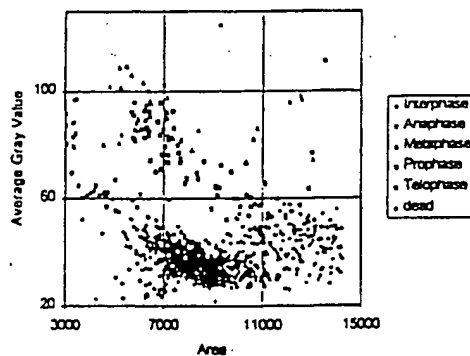


Fig. 8D

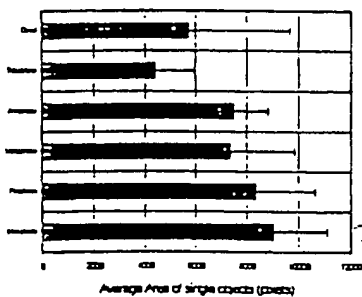


Fig. 8E

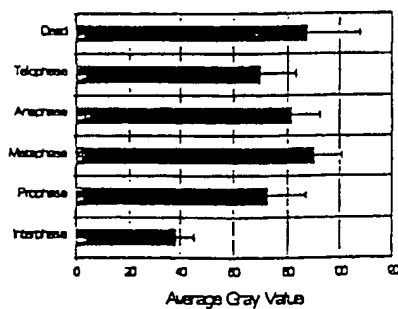


Fig. 8F

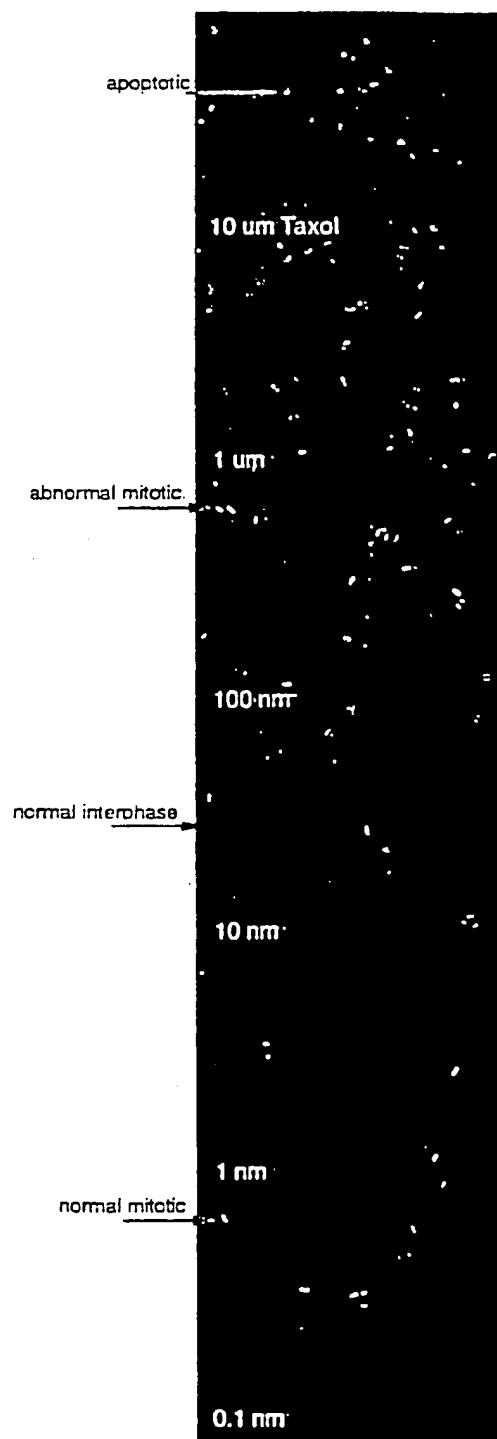


Fig. 9

MDCK cells treated with Taxol for 4.5 hours

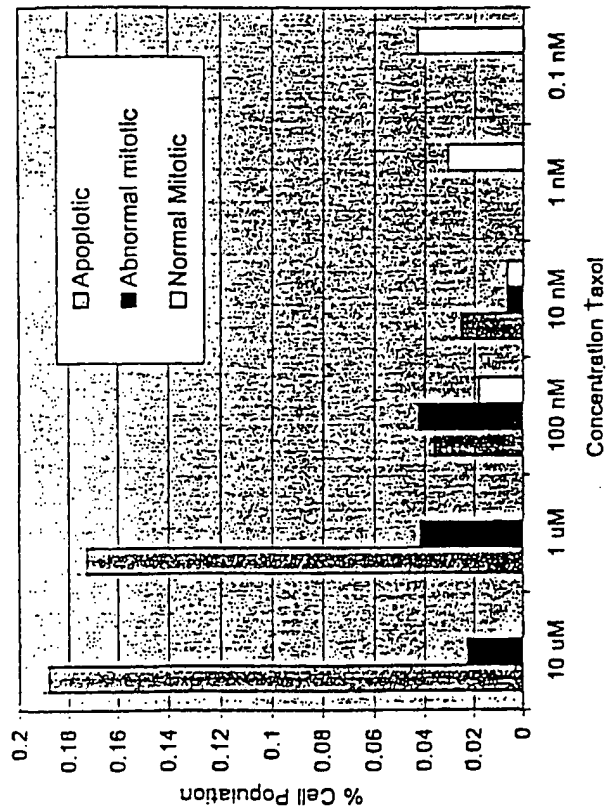


Fig. 10

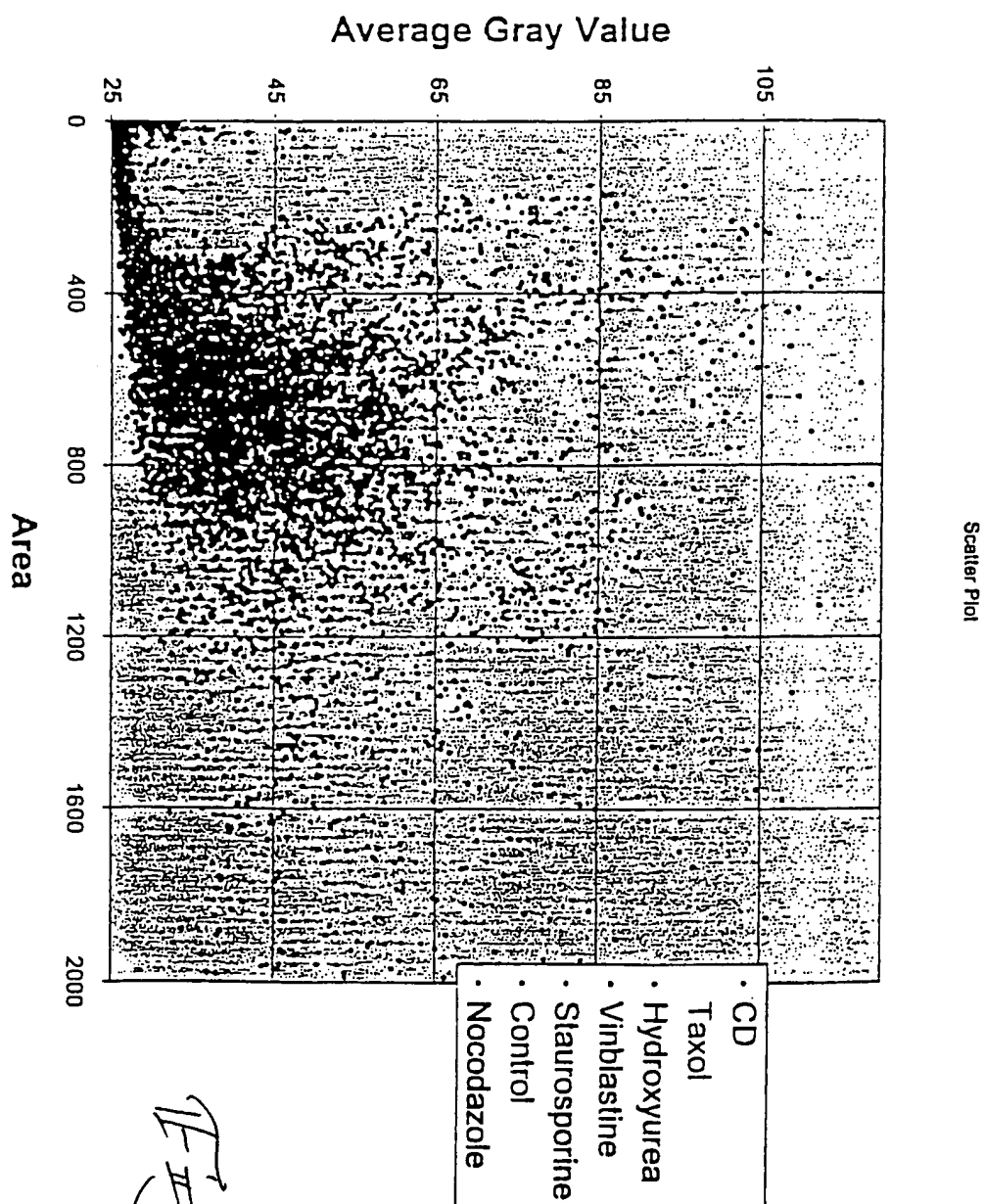
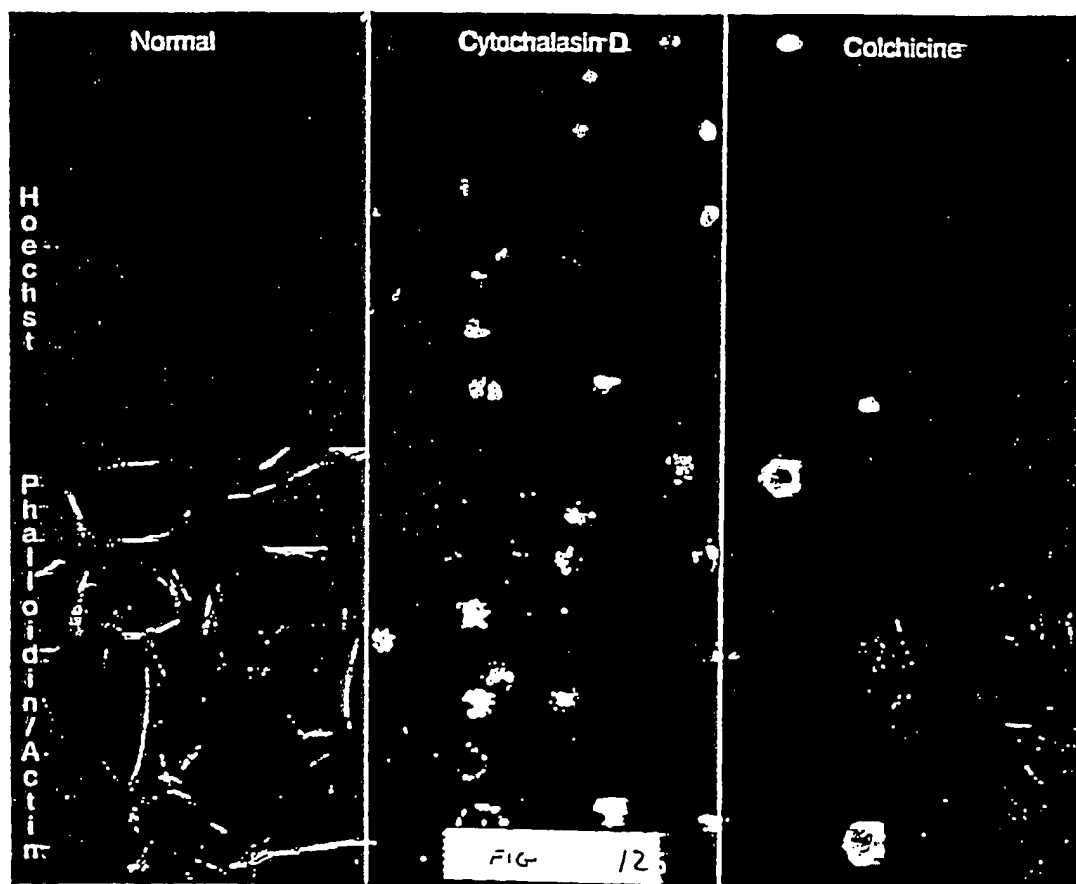


Fig 11



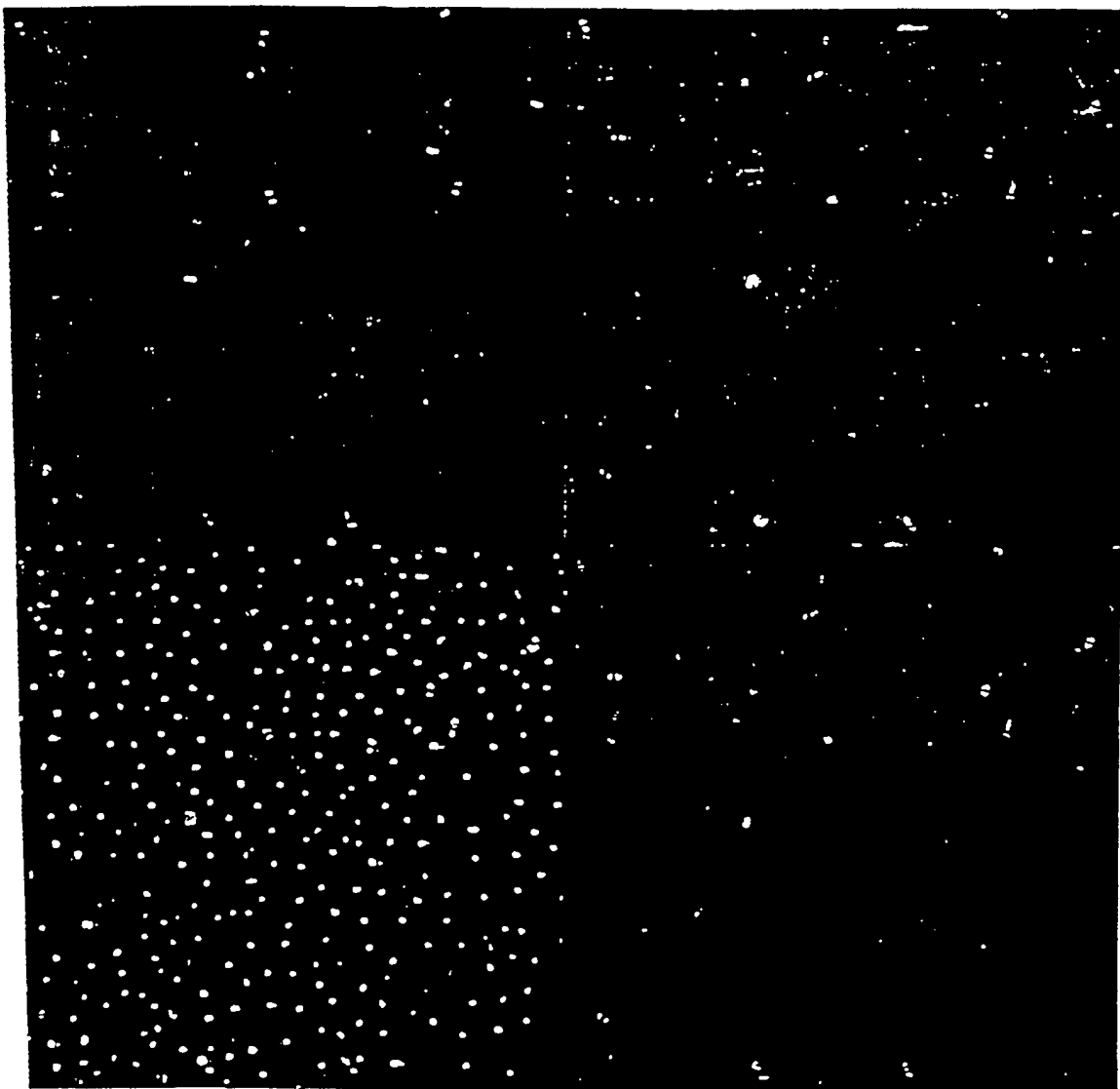


Fig 13

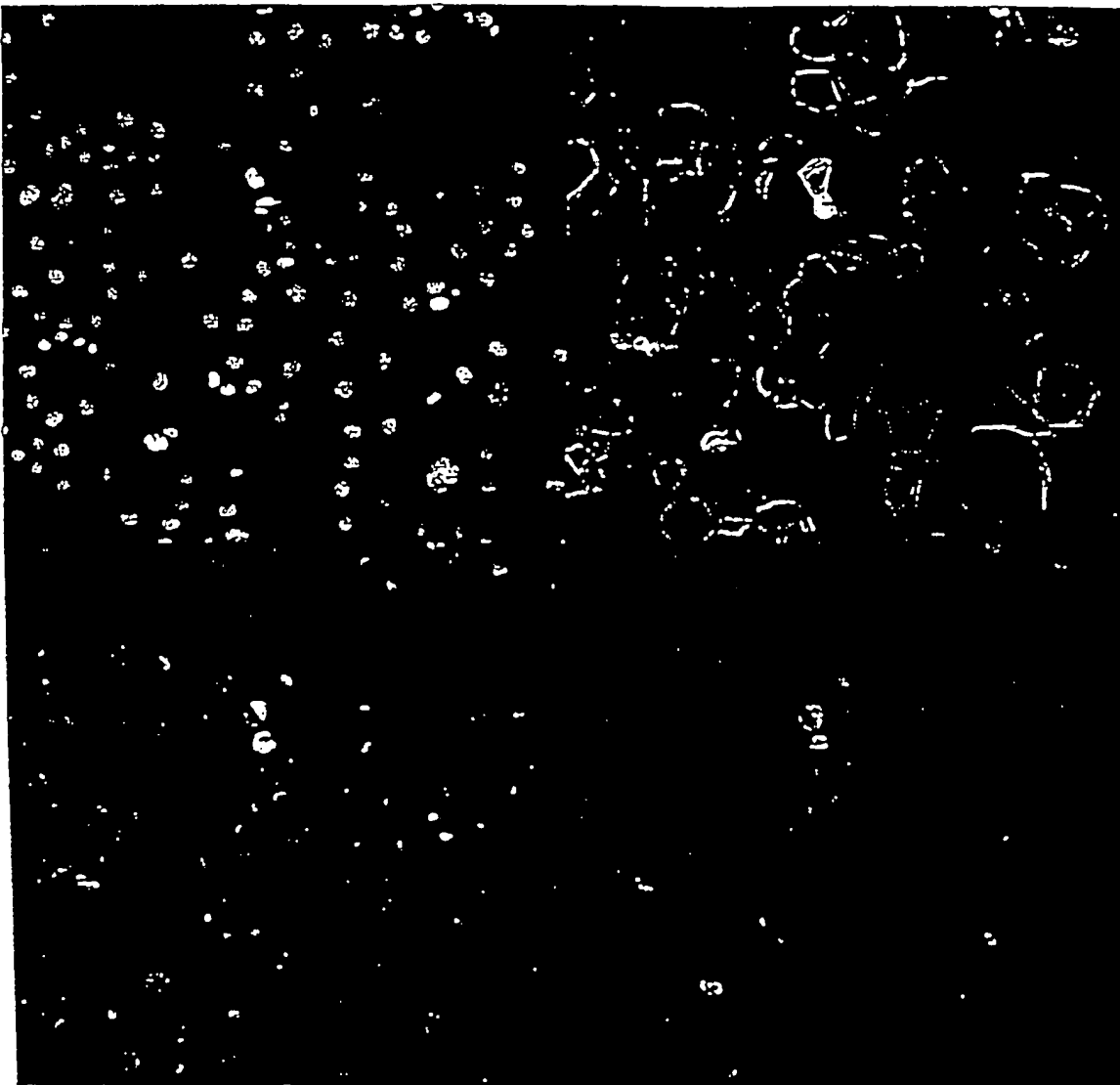


Fig 14

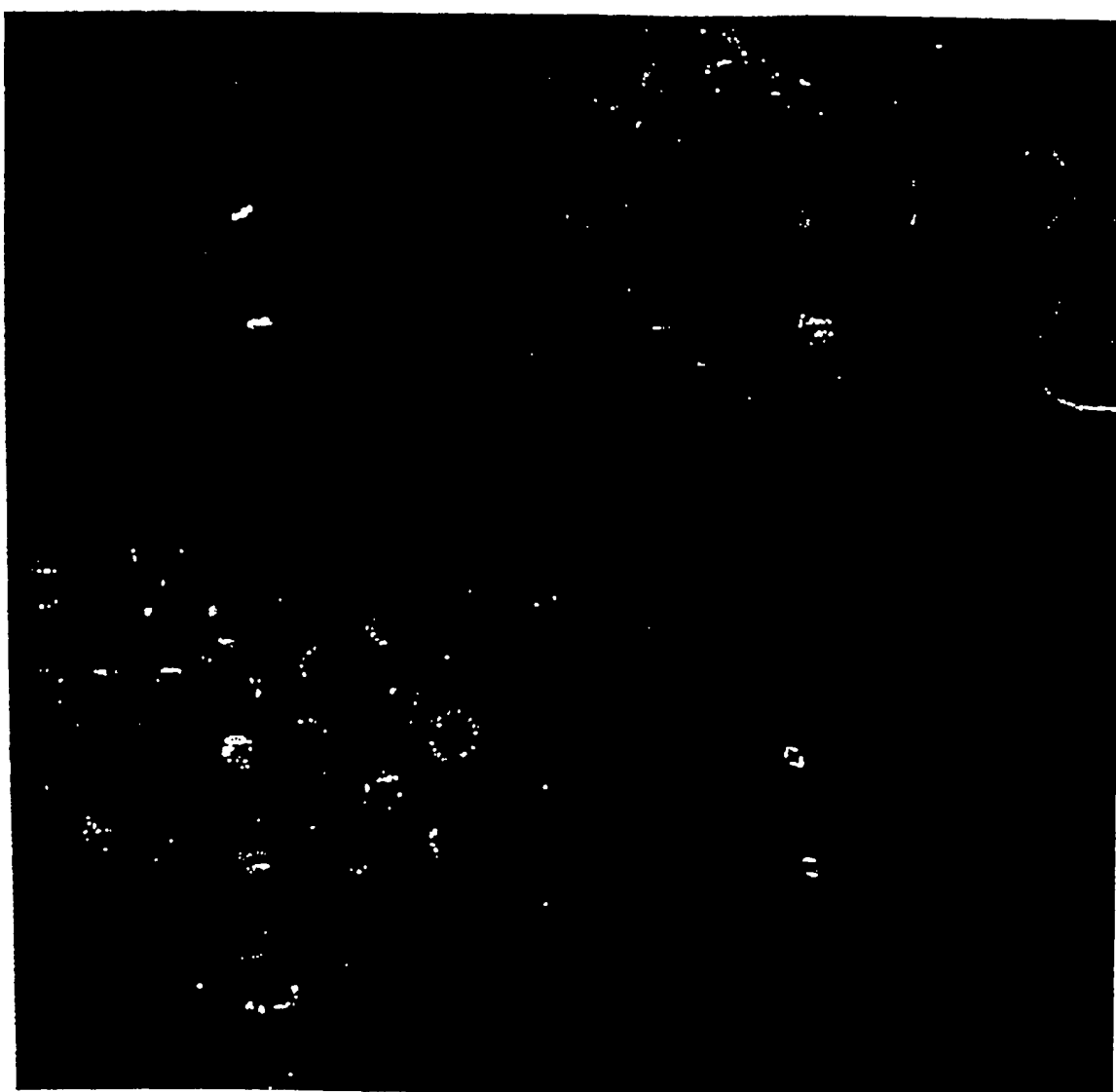


Fig 15

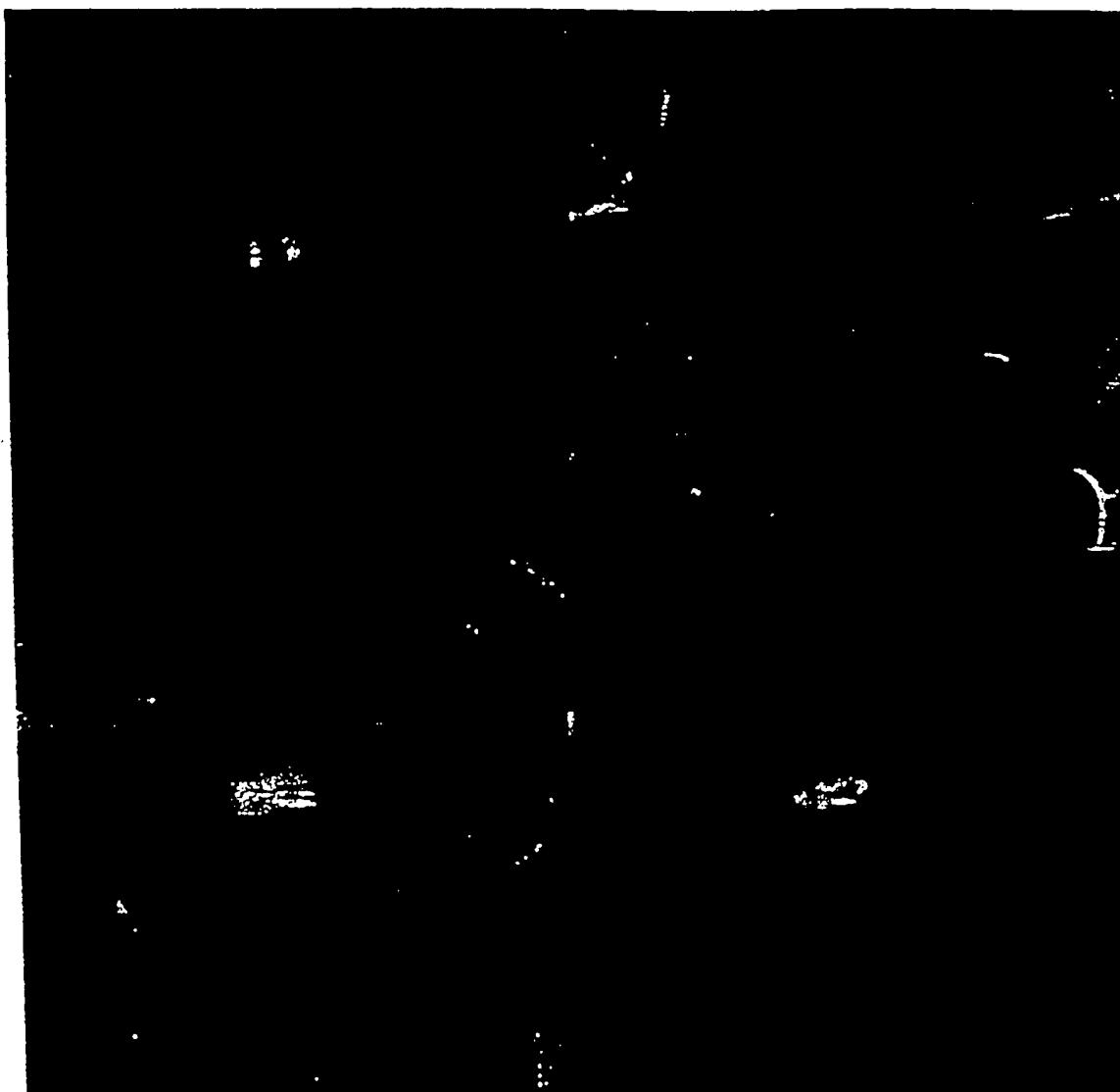


Fig 16

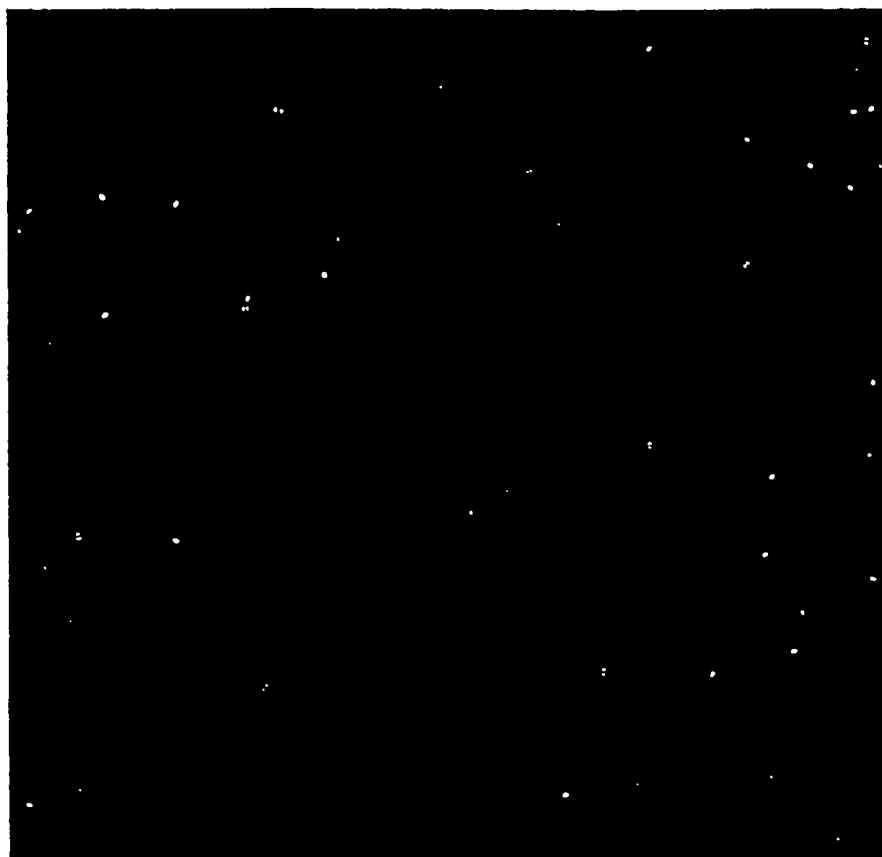


Fig 17

Conversion of morphometric parameters into nucleic acid code
and clustering of the resulting sequences using Neighbor
Joining method.

Compound:	Measurements																							
	Count	Area	Perimeter	Length	Breadth	Fiber length	Fiber breadth	Shape factor	Ell. form factor	Inner radius	Outer radius	Mean radius	Equiv. radius	Equiv. sphere vol.	Equiv. prolate vol.	Equiv. oblate vol.	Equiv. sphere surface area	Average gray value	Total gray value	Optical density	Radial dispersion	Texture Difference Moment	EFA Harmonic 2, Semi-Maj	EFA Harmonic 2, Semi-Min
Control	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	a	t	t
Taxol	a	t	t	t	t	t	t	t	a	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
CD	c	a	a	a	t	a	t	t	c	a	a	a	a	a	a	a	a	t	a	a	a	t	a	g
Nocodazole	c	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
Staurosporine	g	g	c	a	a	t	a	a	t	g	a	a	a	t	g	g	g	a	a	t	a	t	a	a
Vinblastine	c	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	g	t	t	t	t	t	t
Hydroxyurea	g	t	t	t	t	t	t	g	t	t	t	t	t	t	t	t	t	t	t	c	t	a	t	t

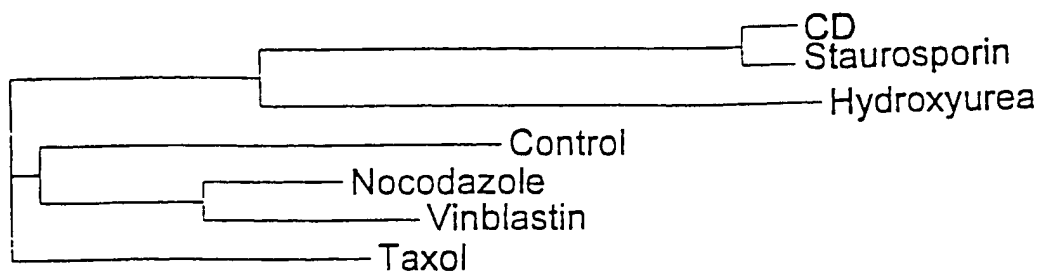


Fig 10

Conversion of morphometric parameters into amino acid codes
and clustering of the resulting sequences using Neighbor
Joining method.

	Count	Area	Perimeter	Length	Breadth	Fiber length	Fiber breadth	Shape factor	Ell. form factor	Inner radius	Outer radius	Mean radius	Equiv. radius	Equiv. sphere vol.	Equiv. prolate vol.	Equiv. oblate vol.	Equiv. sphere surface a	Average gray value	Total gray value	Optical density	Radial dispersion	Texture Difference Mo	IEFA Harmonic 2, Semi-	IEFA Harmonic 2, Semi-
Control	H	P	T	T	Z	S	D	W	F	S	T	T	T	T	C	C	P	P	M	C	T	G	T	T
Taxol	G	F	M	M	P	M	P	H	G	S	M	M	W	C	F	P	F	R	C	M	M	H	M	P
CD	F	G	G	G	M	G	M	K	A	G	G	G	G	G	G	G	G	H	G	G	G	M	G	V
Nocodazole	W	F	M	M	W	M	P	T	R	S	M	M	M	F	M	W	F	M	M	R	M	M	M	F
Staurosporine	N	V	A	G	G	M	G	Y	V	G	G	G	M	V	V	V	V	G	G	H	G	M	G	V
Vinblastine	F	W	W	M	W	W	C	W	D	S	M	W	W	M	M	M	W	M	V	E	M	M	M	F
Hydroxyurea	S	H	H	H	H	H	H	V	H	H	H	H	H	H	H	H	H	H	H	A	H	G	H	D

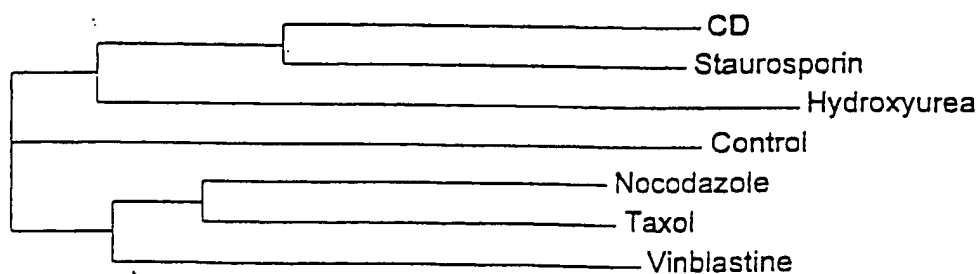


Fig 19

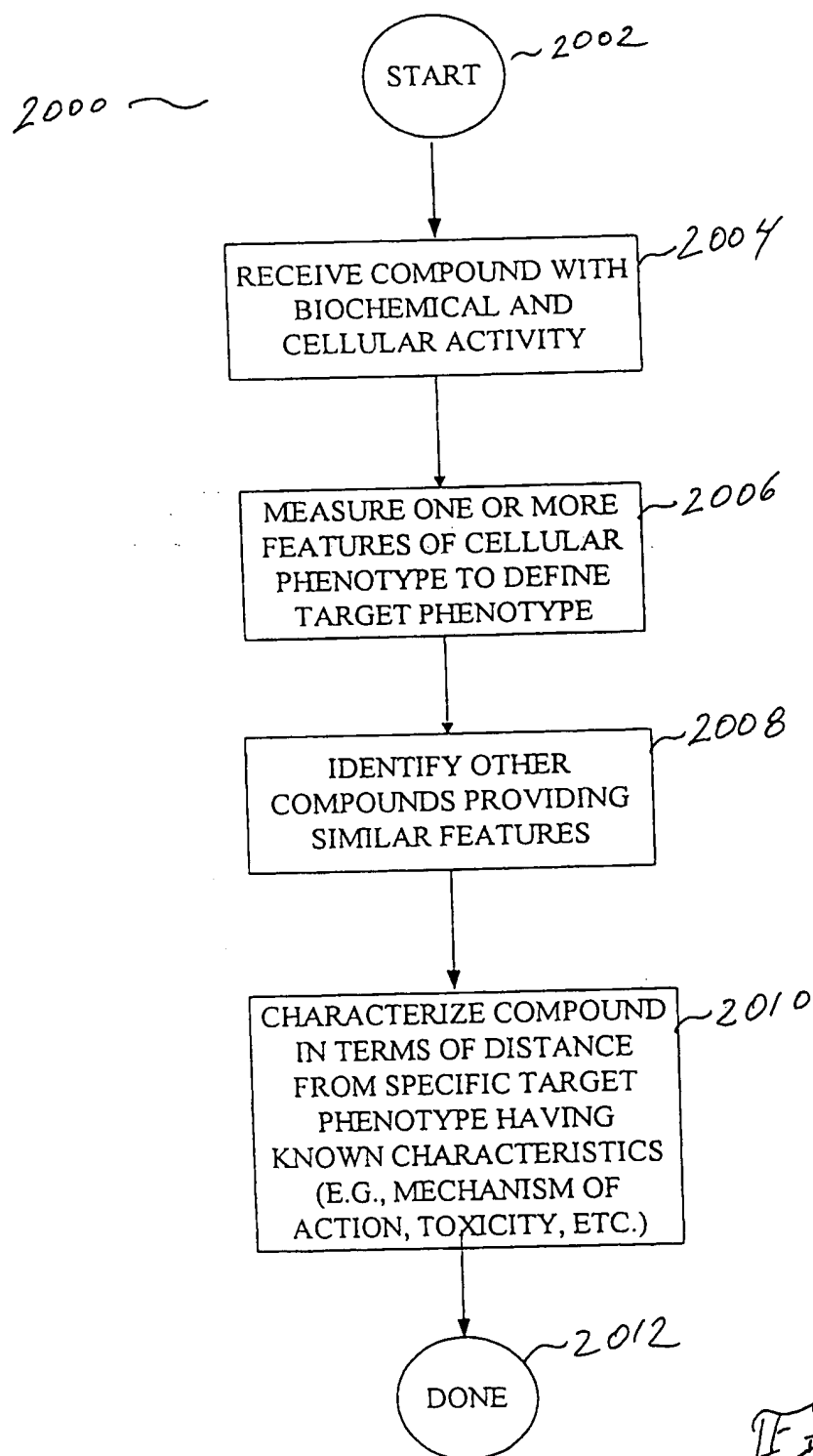


Fig 20

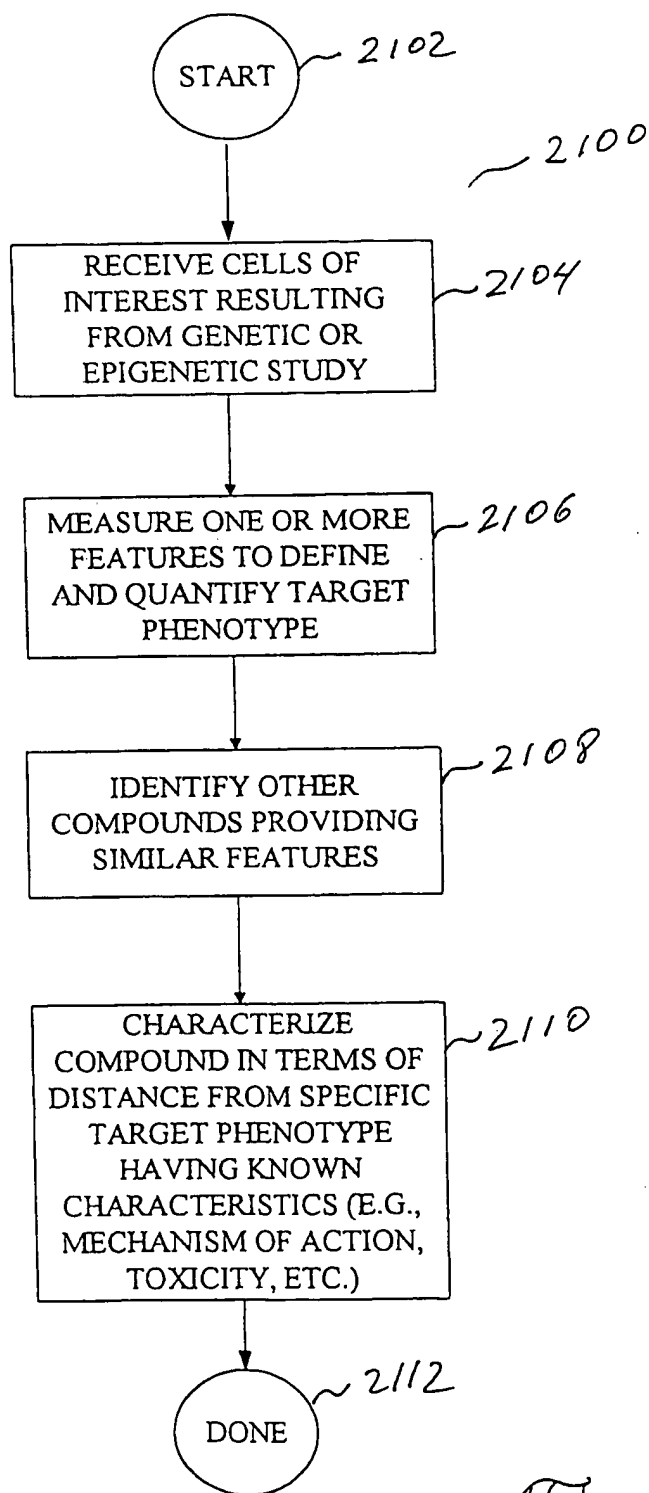


Fig 21

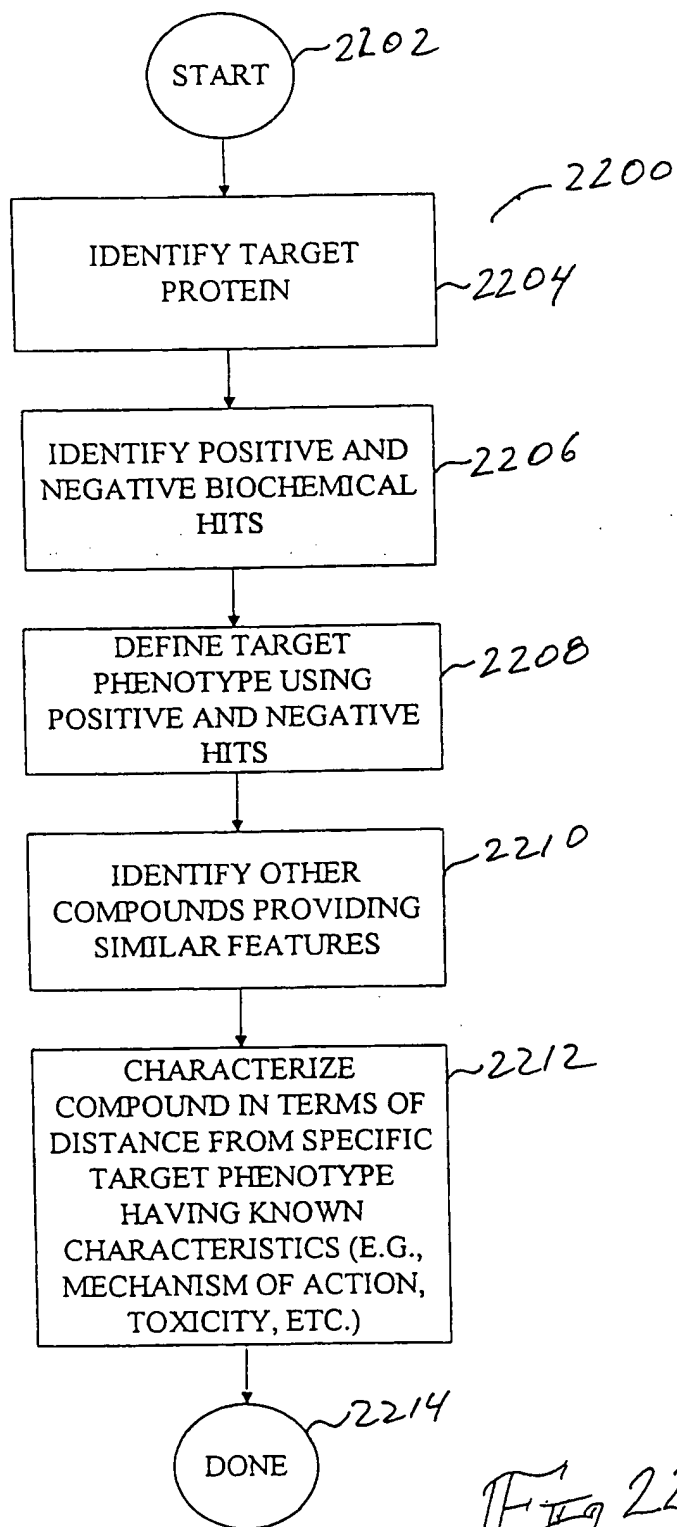


Fig 22

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(72) Inventors: SABRY, James, H.; 52 Buena Vista Terrace, San Francisco, CA 94117 (US). ADAMS, Cynthia, L.; 615 Georgia Avenue, Palo Alto, CA 94306 (US). VAISBERG, Eugeni, A.; 647 Pegasus Lane, Foster City, CA 94404 (US). CROMPTON, Anne, M.; 2 Bellair Place, San Francisco, CA 94133 (US). BLUM, Robert, I.; 17 Shoreview Avenue, San Francisco, CA 94121 (US). OESTREICHER, Donald, R.; 904 Old Town Court, Cupertino, CA 95014-4024 (US). SIGAL, Nolan, H.; 941 Berry Avenue, Los Altos, CA 94024 (US).

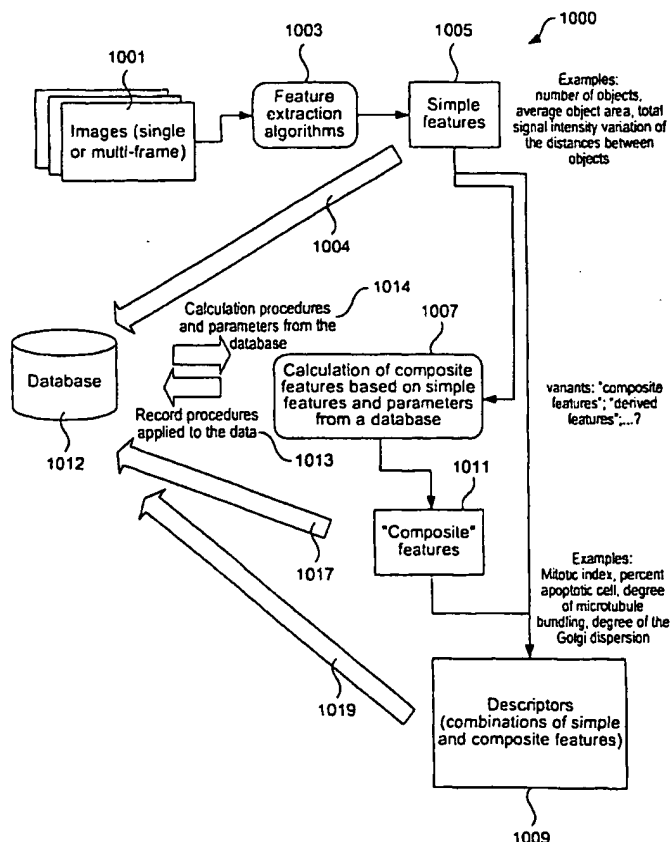
(74) Agent: LOUIE, Michael, L.; Beyer Weaver & Thomas, LLP, P.O. Box 130, Mountain View, CA 94042-0130 (US).

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[Continued on next page]

(54) Title: METHOD AND APPARATUS FOR PREDICTIVE CELLULAR BIOINFORMATICS



(57) Abstract: Techniques for using information technology in therapeutics or drug discovery. In an exemplary embodiment, techniques for determining information about the properties of substances based upon information about structure of living or non-living cells exposed to substances are provided. A method according to the present invention enables researchers and/or scientists to identify promising candidates in the search for new and better medicines or treatments using, for example, a cellular informatics database. The present invention further teaches a system for acquiring knowledge from cellular information. The system has a database 1012 comprising a database management module ("DBMS"). The system also has a variety of modules, including a population module coupled to the DBMS for categorizing and storing a plurality of features (e.g., cell size, distance between cells, cell population, cell type) from an image acquisition device into the database. The system has a translation module coupled to the DBMS for defining a descriptor from a set of selected features from the plurality of features. In a specific embodiment, the descriptor is for a known or unknown compound, e.g., drug. A prediction module is coupled to the DBMS for selecting one of a plurality of descriptors from known and unknown compounds from the database based upon a selected descriptor from a selected compound. The selected compound may be one that is useful for treatment of human beings or the like.



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International Application No

PCT/US 00/13154

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G06F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 38490 A (BIODX INC ;DUNLAY R TERRY (US); GOUGH ALBERT H (US); GIULIANO KENN) 3 September 1998 (1998-09-03) cited in the application	1-6, 24-27
Y	page 1; claims 1-43	7-23
X	WO 98 45704 A (TULLIN SOEREN ;KASPER ALMHOLT (DK); NOVONORDISK AS (DK); SCUDDER K) 15 October 1998 (1998-10-15) abstract; claims 1-3,22,73,80,81,86	1-6, 24-27
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

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PCT/US 00/13154

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>MONTIRONI R ET AL: "COMPUTED CELL CYCLE AND DNA HISTOGRAM ANALYSES IN IMAGE CYTOMETRY IN BREAST CANCER"</p> <p>JOURNAL OF CLINICAL PATHOLOGY, GB, LONDON, vol. 46, no. 9, 1 September 1993 (1993-09-01), pages 795-800, XP000644549</p> <p>ISSN: 0021-9746</p> <p>abstract</p> <p style="text-align: center;">---</p>	7-13
Y	<p>WO 97 40055 A (DOW CHEMICAL CO ; UNIV TEXAS TECH (US)) 30 October 1997 (1997-10-30)</p> <p>page 18, line 26 - line 32</p> <p style="text-align: center;">---</p>	14-23
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P, X	<p>WO 00 17643 A (CELLOMICS INC ; DUNLAY R TERRY (US); GOUGH ALBERT H (US); RUBIN RIC) 30 March 2000 (2000-03-30)</p> <p>the whole document</p> <p style="text-align: center;">---</p>	1-6, 24-27
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A	<p>GIULIANO K A ET AL: "Fluorescent-protein biosensors: new tools for drug discovery"</p> <p>TRENDS IN BIOTECHNOLOGY, GB, ELSEVIER PUBLICATIONS, CAMBRIDGE,</p> <p>vol. 16, no. 3, 1 March 1998 (1998-03-01), pages 135-140, XP004108592</p> <p>ISSN: 0167-7799</p> <p>page 139, left-hand column, paragraph 4</p> <p>-right-hand column, paragraph 3</p> <p style="text-align: center;">-----</p>	1-27

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intel International Application No

PCT/US 00/13154

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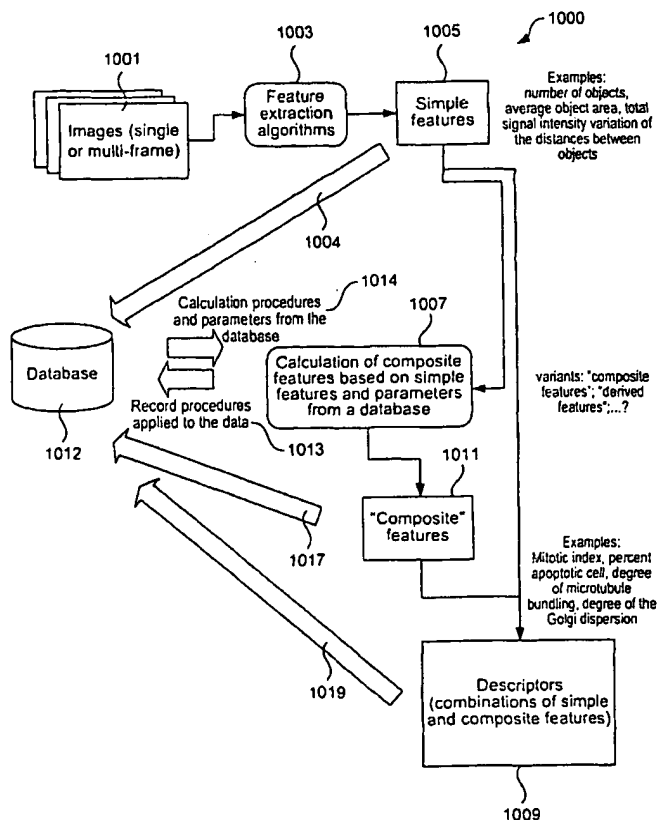
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(71) Applicant: CYTOKINETICS, INC. [US/US]; Suite 2, 280 East Grand Avenue, South San Francisco, CA 94080 (US).

[Continued on next page]

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PATENT APPLICATION
METHOD AND APPARATUS FOR
PREDICTIVE CELLULAR BIOINFORMATICS
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10 computer codes, which may be used to implement aspects of the present invention. Assignee of the present invention reserves all rights with respect to these codes and provides notice herein. Notice is hereby given © Cytokinetics, Inc. 1999.

BACKGROUND OF THE INVENTION

 The present invention provides techniques for information
15 management using a database platform. More particularly, the present invention provides a system including computer code that couples to a database device. The system provides for image capturing of living, dead, or fixed cells or cell fractions used to identify information about substances used on the cells or information about the cells themselves. Accordingly, the present invention can enable researchers and
20 scientists to identify promising candidates in the search for new and better medicines, for example, in drug discovery and development. The principles enumerated herein may, with equal facility, be applied to other applications, including but not limited to use in environmental applications such as determining chemical toxicities and other non-pharmaceutical toxicology uses.

25 For a long time, researchers in the pharmaceutical field have sought for better ways of searching for substances possessing properties that make them suitable as medicines. In the early days, researchers generally relied upon extracts from plants, dyes, and microbiological extracts for such substances. Examples of such substances include the pain reliever aspirin, the anti-cancer drug paclitaxel (brand
30 name TaxolTM), and the heart medication called digoxin. The number of useful medicines has generally been limited.

Purified substances having desirable bio-active properties are also often difficult to discover. Advances in traditional organic chemistry and more recently the rapid chemical synthesis methods often referred to as combinatorial chemistry have increased the number of compounds that researchers test for biological activity. Originally, substances were often initially tested on animals or humans to determine their biological activity. While results from such tests may identify a good drug candidate, they are often time consuming and costly, thus a limited number of substances can be tested. Therefore, pharmaceutical companies have turned to testing their ever-increasing libraries of substances against isolated proteins (drug targets) in biochemical assays that can be carried out at high throughput and low cost. It should be noted that the substances need to be tested in numerous protein tests, each customized for a particular drug target. Therefore, although each protein test may be run at a high-throughput, the design of multiple protein tests can be time-consuming. Substances deemed promising based on results from the protein tests are then tested in lower throughput cellular and animal tests.

There have been some attempts to use image acquisition techniques to screen a large number of substances based upon biological cell information. One such attempt is described in International Application No. WO 98/38490 in the names of Dunlay, et al. Dunlay et al. generally describes a conventional image acquisition system. This conventional system collects and saves images based on certain criteria that are predefined, not on a fixed area of an imaging surface. Additionally, the conventional system has poor lighting design, which makes image processing for multiple cells difficult. Furthermore, the conventional system is not designed for capturing, populating and utilizing a large database design. The conventional system is designed for customized cellular assays, not as a tool for generation of a cellular informatics database. Without such database capabilities the conventional system cannot be used for screening, analyzing, and comparing large quantities of cells from multiple experiments on multiple days in a predictive, efficient and cost effective manner.

What is needed is a rapid assay to assess the activity of compounds against multiple drug targets simultaneously in a cellular context. What is also needed are techniques for finding the effects of substances on cell function based upon searching and analyzing cellular information.

SUMMARY OF THE INVENTION

According to at least one embodiment of the present invention, techniques for determining information about effects of potential substances on cells are provided. In another exemplary embodiment, the present invention provides a novel system including hardware, computer codes, user interfaces, and a database for acquiring, storing and retrieving cellular and substance information. The cells can include living, dead, or fixed cells or fractions of cells. The present invention enables, *inter alia*, researchers and/or scientists to identify promising candidates in the search for new and better medicines or treatments using, for example, a cellular informatics database.

According to the present invention, a computer program for identification and verification of biological properties of substances can include code that causes a sample of a substance to be administered to a cell. The code determines one or more features for two or more cell components, or markers, in the presence of the substance. The code can form one or more descriptors from the features. Descriptors can be formed by combining features of two or more cell components as identified using the markers. The code can then search one or more descriptors obtained from prior administered substances upon cells in order to locate descriptors having a relationship to the descriptors noted for the substance under study. The code predicts properties of the administered substance based upon the properties of the prior administered substances using the relationship between the descriptors. The code can provide for identifying properties of substances based upon effects on cell characteristics. Candidate drug mechanisms of action, potency, specificity, pharmacodynamic, and pharmacokinetic parameters, toxicity, and the like can be used as substance properties.

In a specific embodiment, the present invention provides a system for acquiring knowledge from cellular information. The system has a database comprising a database management module ("DBMS"). The system also has a variety of other modules, including a population module that is coupled to the DBMS and serves to categorize and store a plurality of features (including but not limited to cell size, distance between cells, cell population, as well as sub-cellular features such as organelle location, protein location and sub-cellular constituent location and

movement) from an image acquisition device into the database. The system has a translation module coupled to the DBMS for defining a descriptor from a set of selected features from the plurality of features. In a specific embodiment, the descriptor is for a known or unknown compound, e.g., drug. A prediction module is
5 coupled to the DBMS for selecting one of a plurality of a descriptors from known and unknown compounds from the database based upon a selected descriptor from a selected compound. The selected compound may be one that is useful for treatment of human beings or the like.

In a specific embodiment, the present invention provides a system for
10 populating a database with cellular information. The system includes a cell holder (e.g., multi-well plate, chip, microfluidic assembly, or other cell chamber) comprising a plurality of sites in a spatial orientation. Each of the sites is capable of holding a plurality of cells to be imaged. Note – the light guide is one embodiment, but we don't want to be limited to it.

15 According to one embodiment, the present system also has an illumination apparatus including a liquid light guide operably coupled to the imaging device for highlighting the plurality of cells in a relatively even spatial manner for image capturing and measurement purposes. Still further, the liquid light guide allows sub-elements (e.g., filter, lamp) of the illumination apparatus to be placed at a
20 remote location to prevent mechanical interference of the cell holder during image capturing. Alternative lighting methodologies may, with equal facility, be implemented.

The system also has an image-capturing device (e.g., charge coupled device camera, translation stage, shutter, microscope, software, shutter control) coupled to a
25 computing device (e.g., computer, network computer, work station, analog computing device, on-board image-processor, and laptop). The image-capturing device is adapted to capture at least one image in at least one of the plurality of sites. One some embodiments, multiple images can be captured, where each image represents a different cell component (or portion). The image-capturing device can be adapted to
30 convert the image into a digital representation, which highlights the feature or features of the one site.

A database storage device (e.g., relational database, object oriented database, mixed object oriented database) includes a database management element. The

database is coupled to the image capturing device. In a specific embodiment, the present system includes modules for feature extraction, generation of descriptions, and data preparation and analysis.

In a specific embodiment, the present invention provides a novel
5 system for determining an effect of a manipulation of a cell using one or more image frames. The system has a plate comprising a plurality of sites in a spatial orientation. Each of the sites is capable of holding a plurality of cells to be imaged. The system also has an image capturing device to capture a plurality of images of at least one site from the plurality of sites. The image capturing device is coupled to the computing
10 device. The system also has an image processing device to combine the plurality of images of at least one site or plurality of sites. The image processing device is operably coupled to the plate. An image processing device is also included. The image processing device can be adapted to form a digitized representation of the plurality of images from the site or plurality of sites. Furthermore, the system has a
15 database storage device comprising a database management element. The database can be adapted to retrieve the descriptor or descriptors of the plurality of features from the computing processing device and storing them in a selected manner.

In a specific embodiment, the present invention provides a system for capturing cellular information. The system also has an image acquisition system
20 comprising a charged coupled device camera adapted to capture an image of a plurality of manipulated cells in various stages of the cell cycle. The stages of the cell cycle are currently understood to include interphase, G0 phase, G1 phase, S phase, G2 phase, M phase, prophase, prometaphase, metaphase, anaphase, and telophase. The principles of the present invention specifically contemplate the application thereof on
25 additional cell cycle stages when and if they are identified.

An optical source is coupled to the image acquisition system for highlighting the plurality of manipulated cells in the various stages of the cell cycle. The illumination apparatus provides for an acquisition of the image of the plurality of manipulated cells. In a specific embodiment, the illumination apparatus has a liquid
30 light guide coupled to a light source at a remote location.

A variety of user interfaces are utile for accessing the several features of the present invention. Those having ordinary skill in the art will appreciate that different user interfaces may be required to support different research scenarios. The

present invention specifically contemplates the utilization of a wide variety of user interfaces.

Numerous benefits are achieved by way of the present invention over conventional techniques. The present invention can provide techniques for predictive
5 cellular bioinformatics that can streamline a number of important decisions made in the drug discovery industry. The present invention can be implemented using off the shelf hardware including databases. In other aspects, the present invention can find useful information about substances as well as cells or portions of cells. Furthermore, the present invention can acquire more than one feature using more than one
10 manipulation. Moreover, the present invention can provide information about a wide variety of cellular information that is not conventionally available. This information includes information about different cell components, e.g., nuclei and Golgi apparatus. Still further, the present invention provides an automated or semi-automated technique for acquiring images and populating a database. The present
15 database can be combined with others such as genomics, and the like. Moreover, the present invention can be implemented to predict, *inter alia*, a mechanism of action, toxicity, target validation, and pre-clinical disease model.

A further understanding of the nature and advantages of the invention herein may be realized by reference to the remaining sections of the specification and
20 the attached drawings.

BRIEF DESCRIPTION OF THE DRAWING

For more complete understanding of the present invention, reference is
5 made to the accompanying Drawing in the following Detailed Description of the
Invention. In the drawing:

Fig. 1 is a simplified system diagram according to an embodiment
according to the present invention;

10 Figs. 1A-1B are more detailed diagrams of database systems according
to embodiments of the present invention;

Fig. 2 is a simplified block diagram according to an alternative
embodiment according to the present invention;

Figs. 3-6 are simplified diagrams of system elements according to
embodiments of the present invention;

15 Figs. 7A-7K illustrate representative block diagrams of simplified
process steps in a particular embodiment according to the present invention;

Fig. 8A-8F illustrate representative quantified descriptors of effects of
manipulations on images of cells in a particular experiment;

20 Fig. 9 illustrates example images for different types of morphologies in
a particular experiment;

Fig. 10 illustrates a distribution of various morphologies in a cell
population responsive to drug concentration in a particular experiment;

Fig. 11 illustrates a graph of quantified features of effects of
manipulations on cells in a particular experiment;

25 Fig. 12 illustrates effects of external agents on cells in a particular
experiment;

Fig. 13 illustrates 4 panels for each marker for a plurality of A549 cells
in a particular experiment;

30 Fig. 14 illustrates 4 panels for each marker for a plurality of OVCAR-3
cells in a particular experiment;

Fig. 15 illustrates 4 panels for each marker for a plurality of OVCAR-3
cells at 20x in a particular experiment;

Fig. 16 illustrates 4 panels for each marker for a plurality of OVCAR-3 cells at 40x in a particular experiment;

Fig. 17 illustrates a representative input for a morphometric analysis program in a particular embodiment according to the present invention; and

5 Figs. 18-19 illustrate examples of the generation of pseudo-sequences and clustering in a particular embodiment according to the present invention.

Fig. 20 is a block diagram for a first research scenario;

Fig. 21 is a block diagram for a second research scenario; and

Fig. 22 is a block diagram for a third research scenario.

10 Reference numbers refer to the same or equivalent parts of the invention throughout the several figures of the Drawing.

DETAILED DESCRIPTION OF THE INVENTION

According to the present invention, techniques for determining information about manipulated cells or substances based upon living, fixed, or dead cell structures or portions of cells are provided. In an exemplary embodiment, the present invention provides a novel system including computer codes coupled to a database and user interfaces for acquiring, storing and retrieving such information. Other embodiments provide a novel image capturing system for providing digitized representations of live and dead cell structures or the like.

Fig. 1 is a simplified system diagram 10 of a cellular knowledge-based system according to an embodiment of the present invention. This diagram is merely an example and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. The present system 10 includes a variety of elements such as a computing device 13, which is coupled to an image processor 15 and is coupled to a database 21. The image processor receives information from an image capturing device 17, which image processor and image capturing device are collectively referred to as the imaging system herein. The image capturing device obtains information from a plate 19, which includes a plurality of sites for cells. These cells can be biological cells that are living, fixed, dead, cell fractions, cells in a tissue, and the like. The computing device retrieves the information, which has been digitized, from the image processing device and stores such information into the database. A user interface device 11, which can be a personal computer, a work station, a network computer, a personal digital assistant, or the like, is coupled to the computing device.

Fig. 1A is a simplified diagram of a database system 1000 according to an embodiment of the present invention. This diagram is merely an example and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize many other variations, modifications, and alternatives. Database system 1000 includes a variety of techniques for processing images from biological cells, e.g., fixed, living, and dead cells, and cell portions. As shown, images are acquired 1001. These images can be from a single frame or multiple frames. As merely an example, an image processing system may analyze such images. One example of

such an image processing system is described below, but should not be construed as limiting certain claims.

In a specific embodiment, cell samples are manipulated using a compound (e.g., substance, drug). The cell samples are imaged for a simple portion or portions, e.g., manipulated cell substructure, manipulated spatial feature of cell, cell density. Image processing techniques are used to extract 1003 the feature or features from the image or images. The features can be an independent or a dependent set of cell characteristics (which may be predominately visual) including, for example, count, area, perimeter, length, breadth, fiber length, fiber breadth, shape factor, elliptical form factor, inner radius, outer radius, mean radius, equivalent radius, 10 equivalent sphere volume, equivalent prolate volume, equivalent oblate volume, equivalent sphere surface, average intensity, total intensity, optical density, radial dispersion, texture difference, and others. Each of these features corresponds to a similar manipulation by a compound. Each manipulation forms a new set of features, which are identifiable to the compound. Once each set of features has been extracted, the feature set is populated 1004 into a database 1012. Accordingly, the database includes many sets of features, where each set corresponds to a different manipulation for a selected cell. Each set of features corresponding to a manipulation provides a descriptor 1009, which is also stored 1019 in the database. The descriptor is a "finger print" including each feature for the manipulation. Each descriptor may be unique, or 20 may have similarities to other descriptors or may even be the same as other descriptors for known and unknown manipulations.

The present system retrieves features, which we define as simple features herein, and forms composite features 1007 from them. More than one feature 25 can be combined in a variety of different ways to form these composite features. In particular, the composite feature can be any function or combination of a simple feature and other composite features. The function can be algebraic, logical, sinusoidal, logarithmic, linear, hyperbolic, statistical, and the like. Alternatively, more than one simple feature can be combined in a functional manner (e.g., arithmetic, algebraic). As merely an example, the composite feature equals a sum of 30 feature 1 and feature 2, where these features correspond to the same manipulation. Alternatively, the composite feature equals feature 1 divided by feature 2. Alternatively, the composite feature equals feature 1 minus feature 2. Alternatively,

the composite feature equals a constant times feature 1 plus feature 2. Of course, there are many ways that the composite feature can be defined. The present system also stores 1017 these features in the database. The composite features can also be further combined with simple features. Once these features are defined as descriptors, they are stored 1019 in the database.

Fig. 1B is a simplified diagram of a database system engine 2000 according to an embodiment of the present invention. This diagram is merely an example and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize many other variations, modifications, and alternatives. The engine can be implemented into the present database for populating, searching, and predicting compound or cell characteristics. As merely an example, engine 2001 includes an input/output module 2008. The input/output module is used to input and output information from the database. The information includes, among others, a plurality of feature sets, which correspond to many manipulations. Additionally, the information includes descriptors, which each corresponds to a set of features from the manipulation. The database also has a population module, which is used to configure the features based upon an entity relationship, which has been predetermined.

The database engine also has other modules. In particular, the database has a transcription module, which transfers a preselected set of features and creates a descriptor from them. The transcription module can be used to take a known compound, which has features, to transcribe them into a descriptor. Alternatively, the transcription module can be used to take an unknown compound, which has features, to transcribe them into a descriptor. These descriptors are provided into the database for subsequent use. Finally, the database engine has a prediction module, which can be used to potentially predict a property (e.g., mechanism of action) of an unknown compound. Here, the unknown compound is provided with a descriptor, but the property of the compound is unknown. In one embodiment, the prediction module compares a descriptor of an unknown compound with the many descriptors of known compounds, which were in the populated database. Depending upon the matching criteria, the prediction module will attempt to uncover one or more descriptors of known compounds. Once the prediction module finds the descriptors of the known compounds based upon the descriptor for the unknown compound, it identifies a potential property of such unknown compound for analysis and review. Here, it is

believed that certain features of the known compound, which are similar to those features of the unknown compound may uncover a property to the unknown compound. Details of the present software engine are described more fully below.

Fig. 2 is a simplified block diagram 20 of a cellular knowledge-based system according to an alternative embodiment of the present invention. This diagram is merely an example and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. Like reference numerals are used in the present diagram as the previous diagram for easy cross-referencing, but are not intended to be limiting in any manner.

10 The present diagram 20 includes a variety of elements such as a processor 13 or computing device coupled to a database 11. The processor can be used for retrieving and storing information from the database. The system also includes a plurality of system elements, such as a cleaner 23, a dispenser 25, and an image capturing system 27, which are also coupled to the database in some embodiments. These elements can

15 be coupled to each other through a network or the like. As merely an example, the network can be a NetWareTM network from Novell Corporation or an internet network or the Internet but can also be others and any combination thereof. The system also has an output device 31, which can be used to output information from the database, processor, or other system elements. Details of these elements are described more

20 fully below in reference to the Figs.

Figs. 3-5 are simplified drawings of system elements according to embodiments of the present invention. These diagrams are merely examples and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. As merely an example,

25 Fig. 3 is a simplified diagram of a processor or computing device 13. The computing device 13 includes a bus 112 which interconnects major subsystems such as a central processor 114, a system memory 116 (e.g., random access memory), an input/output ("I/O") controller 118, an external device such as a display screen 124 via a display adapter 126, a keyboard 132 and a mouse 146 via an I/O controller 118, a SCSI host

30 adapter (not shown), and a floppy disk drive 136 operative to receive a floppy disk 138.

The computing device has other features. Storage Interface 134 may act as a storage interface to a fixed disk drive 144 or a CD-ROM player 140 operative

to receive a CD-ROM 142. Fixed disk 144 may be a part of computing device or may be separate and accessed through other interface systems. A network interface 148 may provide a direct connection to a remote server via a telephone link or to the Internet. Network interface 148 may also connect to a local area network ("LAN") or other network interconnecting many computer systems. Many other devices or subsystems (not shown) may be connected in a similar manner. Also, it is not necessary for all of the devices shown in Fig. 3 to be present to practice the present invention, as discussed below. The devices and subsystems may be interconnected in different ways from that shown in Fig. 3. The operation of a computer system such as that shown in Fig. 3 is readily known in the art and is not discussed in detail in this application. Computer code to implement the present invention, may be operably disposed or stored in computer-readable storage media such as system memory 116, fixed disk 144, CD-ROM 140, or floppy disk 138. The computer code can be organized in terms of processes or modules, depending upon the application. That is, the computer code can include a prediction module, a translation, module, or other modules to carryout the functionality described herein, as well as others.

Figs. 4 and 5 are simplified diagrams of an imaging system 200 according to an embodiment of the present invention. As shown, the imaging system 200 includes a variety of features such as housing 203, which holds a stage assembly 204. The stage assembly includes an x-stage movement element 206, which is along an x-direction, and a y-stage movement element 207, which is along a y-direction. The imaging system also includes a z-direction movement element, which is perpendicular to the x-y plane. The z-direction movement motor can be attached to the stage, or to the objective nosepiece by way of the microscope housing, or as an external motor between the objective and the microscope housing. The stage can align in any one of the directions to an accuracy of one micron and less, or one-half micron and less, or one-quarter micron and less, depending upon the embodiment.

The stage holds a plate 202 or cell holder, which houses one of a plurality of samples. The plate includes a spatial array 209 of process sites. Each of the process sites can include a plurality of cells and solutions depending upon the embodiment. Each of the sites can carry a sufficient amount of solution to prevent substantial evaporation of the sample during processing in some embodiments. In embodiments for large scale analysis, the plate includes at least 96 sites, or more than

or equal to 384 sites, or more than or equal to 1,536 sites. The plate bottom is transparent and thin, which allows light to pass through the sample. Additionally, the plate is made of a suitable chemical resistant material. As merely an example, the plate can be either a 96, or 384, or 1536 or other formats from places such as Becton Dickinson of Franklin Lakes, NJ, or Corning Science Products of Corning, NY. In a preferred embodiment, the plate is a Corning Costar black-walled 96 well plate catalog #3904 from Corning Science Products of Corning, NY, but should not be limited to these in some applications, but can be others.

Also shown is the condenser for the microscope 201, which can be used to collect phase, DIC, or bright field images of the cells. Images resulting from the illumination of the samples to fluorescence, phase, DIC, or bright field techniques are collected using an image capturing device 208, which captures an image or images of cells from the plate. In a specific embodiment, the microscope is an inverted configuration with the objectives on the bottom of the plate and the condenser disposed overlying an upper surface of the sites, while the image capturing device underlies the sites. Images captured by the imaging device, whether analogue or digital, are viewed by a monitor or other devices. The image capturing device can be any camera assembly such as a charge coupled device camera, which is known as a CCD camera, or other high resolution camera capable of capturing images from the sites. In a specific embodiment, the camera is an interline CCD camera which does not require an external shutter.

In a specific embodiment, the present imaging system can be any suitable unit that is flexible for automated image collection using multi-well plastic plates. The imaging system also should be adapted to collect high-resolution images of cells on plastic or glass plates, cell growth chambers, or coverslips. The system also can be used for imaging multiple cell markers in multiple imaging conditions. To accomplish this, the microscope system has a variety of elements such as a light source, a motorized excitation filter wheel and shutter, x-y-z-motorized stage, excitation and emission filters, Fluor phase and DIC objectives, motorized objective nosepiece, dichroic filters, motorized dichroic filter cubes, phase and DIC rings and prisms, CCD camera, and software control. As merely an example, the present imaging system can have components such as those listed in the Table below.

DESCRIPTION	MAKER	MODEL
Microscope	Zeiss	100M
(x-y) motorized stage	Prior	
Xenon lamp	Sutter	Lambda
Filter wheel	Sutter	Lambda-10
Microtitre Plate holder	Prior	500-H223R
Isolation Table	Kinetic Systems	9101-24-85
Objective Spacers	Polytec PI	P-721.90
Camera	Hamamatsu	C47-95
Computer	IBM	IntelliStation
Software	Metamorph	v.4
Objectives	Zeiss	Achroplan 10x/0.25 LD-Achroplan 20x/0.4 LD-Achroplan 40x/0.6

Table: Image Acquisition System Elements

5 In a specific embodiment, the present system has the following capabilities, which are not intended to be limiting.

Image acquisition

1) Ability to automatically acquire multi-wavelength images from multiple sites on one multi-well plate, to sequentially name image files, and to log any
10 imaging parameter information with image files.

2) Ability to link images with a larger database/spreadsheet of information.

3) Ability to automatically collect multiple plates by interfacing the imaging system with a robotic arm.

15

X-Y control

1) Ability to place 96, 384, or 1536 well plates onto microscope stage and move to each well sequentially.

2) Ability to return to each well and collect another round of images (multi-site time-lapse) or ability to collect rapid time-lapse information at each well (time-lapse of many wells).

3) Ability to collect a low magnification image, automatically determine features which may be of interest, automatically change the objective to a higher magnification, and collect high magnification images of a fixed number of those identified cells in the sample.

4) Ability to collect multiple frames in each site.

10 Z control

1. Ability to auto-focus with substantially minimal damage to biological specimen or fluorophore.

2. Ability to auto-focus rapidly.

15 The present embodiment of the imaging system is shown by way of Figs. 5A and 5B. These diagrams are merely examples and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. The present imaging system 40 includes a variety of elements such as a microscope 41, which is preferably an epi-fluorescent microscope, but can be confocal, multiphoton, or hybrid types. The microscope includes elements
20 41A, the motorized Z-axis; 41B, the motorized dichroic filter cube holder; and 41C, the motorized objective nosepiece. In one embodiment, the microscope is a Model 100M made by Zeiss. The microscope communicates to computer 51 through control lines 73, 75, and 76. The imaging system also has camera 50 coupled to controller
25 50A and computing device 51, which oversees and controls operations of the elements of the imaging system.

The present microscope includes drivers for spatially moving a stage in two dimensions, including an x-direction, a y-direction, and moving the objective nosepiece in a z-direction in a Cartesian coordinate system. The z-direction
30 movement is provided using a fast z-motor, which can make z-direction adjustments within a predetermined time. The z-direction movement generally provides for focussing of the sample to the camera. The focussing occurs within the predetermined time of preferably ten seconds and less, or five seconds and less, or one

second and less, depending upon the embodiment. As merely an example, the z-motor or positioner can be a model PIFOC objective nanopositioner made by a company called Physik Instrumente of Waldbronn, Germany, but also can be others. The z-motor couples to computer 51 through line 63, which may also include a
5 controller. Depending upon the embodiment, a second z-motor 41A connected to the computer 51 by line 73 may be used to keep the z-motor 42 in the center of its travel. Alternatively, in other embodiments the stage could be provided with a z-motor allowing for movement of the stage in the z-direction.

The present stage also includes an x-y stage 43. The x-y stage moves
10 plate 59, e.g., 96 site, 384 site, 1536 site. The x-y stage moves plate in an x-y spatial manner. The stage has an accuracy or repeatability of about 1 micron and less, or about 2 microns and less. The stage can move in a continuous manner or a stepped manner. The stage also can move up to 30 mm/sec. or faster. The stage also can move 1 mm/sec. and less, depending upon the embodiment. The stage can also step
15 0.1 micron and less or 1 micron and less, as well as other spatial dimensions. The stage can be one such as a Proscan Series made by Prior Scientific of Rockland, MA but can also be others. The stage is controlled via control line 61 through controller 43A, which couples to computer 51 through control line 65.

The stage includes plate holder 44. The plate holder can hold a single
20 plate. In other embodiments, plate holder can also hold multiple plates. The plate holder can use mechanical, electrical, fluid, vacuum and other means for holding the plate or plates. The plate holder also is sufficiently stable for securing the plate. As merely an example, the plate holder is a Model 500-H223R made by Prior Scientific of Rockland, MA. In some embodiments, the plate holder may need adjustment in
25 the z-direction to provide for a desirable focus of a sample on a plate. In these embodiments, the plate holder is supported by spacers 45 or a plurality of stage pins, which mechanically elevate the plate holder in the z-direction. These pins are generally made of a suitable material for supporting such plate holder and also are sufficiently resistant to chemicals and the like.

30 In some embodiments, the entire imaging system is placed on an isolation table 49. The isolation table is disposed between the microscope and support structure. The isolation table is designed to prevent excessive vibration of the plate. The isolation table is made of a suitable material such as steel and honeycomb but can

be others. The table has a thickness of about 8 inches or preferably less than about 24 inches. In one embodiment, the table is Model 9101-24-85 made by Kinetic Systems of Boston, MA.

The imaging system also has a lamp or illumination assembly 62. The lamp assembly provides for a light source (See reference letter B) to a plurality of elements in the imaging system. For easy reading, the light path is defined by the dotted lines, which are not intended to be limiting. The lamp assembly has a variety of elements such as a Xenon lamp 46. The Xenon lamp provides light at about 320 to 700 nanometers (Prefocused). The Xenon lamp is 175 or 300 Watts. As merely an example, the lamp can be a Lambda Model made by Sutter Instrument Company of Novato, CA.

Referring to Fig. 5B, the lamp assembly also has a cold mirror 58, an excitation filter wheel 48, excitation filter(s) 55, and an excitation light shutter 57. As shown, light is derived from the Xenon lamp, reflects off of the cold mirror 58, traverses through the excitation filter or filters 55, and is controlled by the excitation light shutter 57. The lamp assembly has filter wheel 48, which houses one of a plurality of filters, including excitation filters. The shutter and filter wheel are controlled via control lines 67, which are coupled to a computer 51 or other type of computing device. The control lines 67 are coupled through controller 57A (for element 57) and controller 48A (for element 48) via control line 69 to computer 51.

Preferably, light traverses from the lamp assembly through a light guide 47 to illuminate features within the plate. The light guide is suitably selected to have a flexible member, which can be used to place lamp source at a remote location away from the imaging device. The flexible member substantially keeps any vibration from the lamp assembly away from the imaging device. In some embodiments, the member is at least 1 foot away from the imaging device. The light guide is a guide, which is a flexible hose-type sleeve. The sleeve is filled with a liquid such as an aqueous solution containing chloride or phosphate. A thin layer may be formed on the inside of the sleeve. The layer can be a containing tetrafluoroethylene and hexafluoropropylene, or containing tetrafluoroethylene and perfluoromethyl vinyl ether, or tetrafluoroethylene and perfluoropropyl vinyl ether. An example of such a light guide is described in International Application No. WO/98/38537 filed February 29, 1997, and assigned to NATH, Gunther. The liquid

light guide has less than about 30% transmission loss of the light at a remote location such as the imaging system.

Light is derived from the lamp assembly and directs off of filter 56, which directs the light upward. Filter 56 can be a dichroic and emission filter, as well as others. The light traverses through microscope nosepiece 41C, and traverses
5 through objective spacers 54. An objective 53 magnifies the light toward a predetermined point on the plate 59. The objective can be, for example, made by Zeiss of Jena, Germany, as well as other companies. The objective can be one of a plurality including 1X, 10X, 20X, 40X, and others, depending upon the application.
10 Magnification can be further expanded or contracted by intermediate optics between the objective and the camera. Selection of filter or filters is controlled by computer 51 via control line 75.

The camera 50 captures an image of cells from plate 59. The image is obtained from light scattering off of cells or portions of cells in the plate through
15 objective 53, through objective spacers, through filters 56, which are captured at camera 50. In this preferred embodiment, the camera is a digital camera, but can be an analogue camera. The digital camera is a CCD camera, which has 1280 by 1024 pixels, or more or less. The pixels can be 6.7 microns in dimension or more or less. The camera preferably is substantially free from an external shutter to quickly capture
20 a plurality of images of cells from the plate. The camera is controlled via control line 71 through controller 50A, which connects to computer 51 through control line 70. The present invention can also include other types of image acquisition devices selected from at least an epifluorescence, a confocal, a total-internal reflection, a phase, a Hoffman, a bright field, a dark field, a differential interference contrast, an
25 interference reflection, or multi-photon illumination device.

The present imaging system stores images on a high density memory device 60. The high density memory device is preferably optical, but can also be magnetic. The high density memory device can be any suitable unit that is capable of storing a plurality of images from a plurality of sites in the plate. The memory device
30 can be a compact disk, which would generally use a compact disk burner or the like. Depending upon the embodiment, the high density memory device is used to archive the images that are captured from the camera in the imaging system. Further details

of the imaging system can be found throughout the present specification, and more particularly below.

As merely an example, the present invention can be implemented using the following sequence of steps, which have been described in a journal entry form.

- 5 Here, images are opened and objects are identified based on a background value that has been edited in starting image acquisition. Information is maintained in a spreadsheet or other database format, which has the following information for each object:

Image Name	Image Plane	Image Date and Time
Elapsed Time	Object #	Total area
Pixel area	Area	Hole area
Relative hole area	Standard area count	Perimeter
Length	Breadth	Fiber length
Fiber breadth	Shape factor	Ell. form factor
Inner radius	Outer radius	Mean radius
Average gray value	Total gray value	Optical density
Radial dispersion	Texture Difference Moment	EFA Harmonic 2, Semi-Major Axis
EFA Harmonic 2, Semi-Minor Axis	EFA Harmonic 2, Semi-Major Axis Angle	EFA Harmonic 2, Ellipse Area
EFA Harmonic 2, Axial Ratio	EFA Harmonic 3, Semi-Minor Axis	

10

After computations are done, the log file is saved. In particular, the file is saved in an appropriate place with an appropriate name.

In a specific embodiment, the present invention provides the following detailed example of journal entries, which should not limit the scope of the invention.

Set Up Sequential File Names	Interactive: user sets up prefix name and image storage directory
Open Data Log	Opens a DDE (Excel) File
Annotate Log File	Interactive: experimental information that will go into the first line of the log file of stage positions
Stage (Go to Origin)	Origin is set as the center of well A1
Stage (Move to Absolute Position)	Offset to upper left hand corner of well (1410, 1621)
Stage (Log Position)	
Stage (Scan Wells)	User picks wells to scan: runs 3x3 image collection.jnl.

3X3 IMAGE COLLECTION.jnl

Stage (Scan)	Takes 9 images of well, -1600 motor steps apart from left to right 3 columns and 3 rows, runs FOCUS, COLLECT IMAGE, SAVE SEQUENTIAL FILE NAME.JNL.
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5

FOCUS, COLLECT IMAGE, SAVE SEQUENTIAL FILE NAME.jnl.

Stage (Log Position)	Logs stage position of each image
ADC – Focus	Opens up the manual focusing window with whatever focus time is current set
Show Message and Wait	Interactive: user hits enter to continue when done focusing

ADC-Acquire from Digital Camera	Takes Hoechst image
Save Using Sequential File Names	
Close	Closes image window

START IMAGE ANALYSIS.jnl

Low Pass	3x3 convolution of already opened image
Low Pass	3x3
Show Region Statistics	Interactive: Show entire image statistics. Calculate background subtraction value for step 4. by: INTENSITY Average + INTENSITY Std. Dev.
Arithmetic	Interactive: User inputs subtraction value from 3. into the constant Value field
Threshold image	Creates threshold 1 unit above 0 to 4096
Integrated Morphometry – Load State	Loads Start Image Analysis.ima Classifier 100 < area < 200000
Integrated Morphometry – Measure	Interactive: Shows area summary information about all objects. The average number is used as the Standard Area in 8.
Object Standards - Set Object Standards	Interactive: User inputs average area value from 7. into Standard Area box to be used by automated IMA for all images

IMA OBJECTS.jnl

Low Pass	3x3 convolution
Low Pass	3x3 convolution
Arithmetic	This background subtraction value needs to be manually entered into this journal from the value determined in START IMAGE ANALYSIS.jnl step 3
Threshold Image	1 unit above 0
Integrated Morphometry – Load State	Hoechst.IMA Classifier 200 < area < 200000
Integrated Morphometry – Measure	Measures statistical info for all objects
Run Journal	Runs log obj and sum data.jnl

Log obj and sum data.jnl

Integrated Morphometry – Log Data	Logs object data into Sheet 1
Integrated Morphometry – Log Data	Log summary data into Sheet 2

5

COLLECT AUTOMATED IMA DATA IN ONE SPREADSHEET.jnl

Run Journal	Runs OPEN OBJECT LOG DDE FILE.JNL
Loop for all Images in a Directory	Loops IMA OBJECTS.jnl
Close Summary Log	
Close Object Log	User must manually save Excel spreadsheet

OPEN OBJECT LOG DDE FILE.jnl

Open Object Log	Opens a DDE object log into sheet 1 of an Excel spreadsheet
Open Summary Log	Opens a summary log into sheet 2

COLLECT AUTOMATED IMA DATA IN ONE SPREADSHEET 16 BIT IMAGES.jnl

Arithmetic	Interactive: Opens Arithmetic window for user to input background subtraction level from START IMAGE ANALYSIS.jnl step 3
Run Journal	Runs OPEN OBJECT LOG DDE FILE.JNL
Loop for all Images in a Directory	Interactive: Runs IMA OBJECTS 16 bit.jnl. User picks directory from which to choose.

5

IMA OBJECTS 16bit.jnl

Low Pass	3x3 convolution
Low Pass	3x3 convolution
Copy to 8-bit Image	No autoscale, to new untitled image
Save Using Sequential File Name	Saves 8bit image using previously defined Sequential File names.
Arithmetic	This background subtraction value needs to be manually entered into this journal from the value determined in START IMAGE ANALYSIS16 TO 8 BIT.jnl step 5
Threshold Image	1 unit above 0 to 255

Integrated Morphometry – Load State	Hoecsht.IMA Classifier 200 < area < 200000
Integrated Morphometry – Measure	Measures statistical info for all objects
Run Journal	Runs log obj and sum data.jnl

START IMAGE ANALYSIS 16 to 8 BIT.jnl

Copy to 8-bit Image	No autoscale, to new untitled image
Close	Closes 16 bit image
Low Pass	3x3 convolution
Low Pass	3x3 convolution
Show Region Statistics	Interactive: Show entire image statistics. Calculate background subtraction value for step 6. by: INTENSITY Average + INTENSITY Std. Dev.
Arithmetic	Interactive: User inputs subtraction value from 5. into the constant Value field
Threshold image	Creates threshold by 1 unit above 0 to 255
Integrated Morphometry – Load State	Loads Start Image Analysis.ima Classifier 100 < area < 200000
Integrated Morphometry – Measure	Interactive: Shows area summary information about all objects. The average number is used as the Standard Area in 10.
Object Standards - Set Object Standards	Interactive: User inputs average area value from 9. into Standard Area box to be used by automated IMA for all images

IMA OBJECTS WITH NEW LOG FILE.jnl

Run Journal	OPEN OBJECT LOG DDE FILE.JNL
Run Journal	IMA OBJECTS.jnl
Close Summary Log	
Close Object Log	User must manually save every Excel spreadsheet generated.

INTERACTIVE IMA OBJECTS.jnl

Threshold Image	User manually sets threshold
Integrated Morphometry – Load State	Hoechst.IMA Classifier 200 < area < 200000
Integrated Morphometry – Measure	Objects
Integrated Morphometry – Log Data	Into open object.log file

5

COLLECT INTERACTIVE IMA DATA.jnl

Close Object Lo g	
Open Object Log	Interactive
Annotate Log File	Interactive: experimental information that will go into the first line of the object log file
Loop for all Images in Directory	Runs INTERACTIVE IMA OBJECTS.jnl

CHANGE FILTER, COLLECT IMAGE. SAVE SEQUENTIAL FILE
NAME.jnl

Stage (Log Position)	
ADC-Focus	

Show Message and Wait	Interactive – user presses Enter when done focusing
ADC – Acquire from Digital Camera	Hoechst
Save Using Sequential File Name	
Close	Close open image

COLLECT HOECHST AND FITC.jnl

Run Journal	FOCUS, COLLECT IMAGE, SAVE SEQUENTIAL FILE NAME.JNL
Run Journal	CHANGE FILTER, COLLECT IMAGE, SAVE SEQUENTIAL FILE NAME.jnl

3X3 IMAGE COLLECTION HOECHST FITC.jnl

Stage (Scan)	COLLECT HOECHST AND FITC.jnl
--------------	------------------------------

5

AUTOMATED 3X3 IMAGE COLLECTION HOECHST FITC.jnl

Set Up Sequential File Names	Interactive: user sets up prefix name and image storage directory
Open Data Log	Excel DDL files
Annotate Log File	Interactive: experimental information that will go into the first line of the log file of stage positions
Stage (Go to Origin)	Origin is set as the center of well A1
Stage (Move to Absolute Position)	Offset to upper left hand corner of well (1410, 1621)

Stage (Log Position)	
Stage (Scan Wells)	Interactive: user picks wells to scan: runs 3X3 IMAGE COLLECTION HOECHST FITC.jnl

AUTOMATED IMAGE COLLECTION.jnl

Set Up Sequential File Names	Interactive: user sets up prefix name and image storage directory
Open Data Log	Opens a DDE (Excel) File
Annotate Log File	Interactive: experimental information that will go into the first line of the log file of stage positions
Stage (Go to Origin)	Origin is set as the center of well A1
Stage (Log Position)	
Stage (Scan Wells)	Interactive: user picks wells to scan: runs FOCUS, COLLECT IMAGE, SAVE SEQUENTIAL FILE NAME.JNL. Well to well travel = (-9035, -9035)

5

STARTUP.jnl

Install and Configure Devices	Open Stage Meta Devices
Set Live Video Channel	

Preferences	<u>Measure Objects</u> : Draw failed classifier objects, Exclude objects that touch the edge of the image, Enable Elliptical Fourier Parameters, turn off Warn users when measurement data will be erased <u>Image Saving</u> : Save Tiff/stk using LZW compression <u>Image Windows</u> : Use transparent thresholds.
Configure Default Paths	C:\Metamorph Data C:\Metamorph Data\Commmon Settings
Load Journal Taskbar	Common.JTB

Nested Journals

Automated 3x3 Image Collection

5 *Loop* 3x3 image collection
 Loop focus, collect image, save sequential file name

Automated 3x3 image collection Hoechst FITC

10 *Loop* 3x3 image collection Hoechst FITC
 loop Collect Hoechst and FITC
 focus, collect image, save sequential file name
 change filter, collect image, save sequential file name

Automated image collection

15 *Loop* focus, collect image, save sequential file name

Collect automated IMA data in one Spreadsheet

Open object log DDE file

Loop IMA objects

Log obj and sum data

Collect automated IMA data in one spreadsheet 16 bit images

5 Open object log DDE file

Loop IMA objects 16 bit

Log obj and sum data

Although the above has been generally described in terms of a specific
10 user interface and software code, other user interfaces and code can also be used. One
of ordinary skill in the art would recognize many other variations, alternatives, and
modifications.

Fig. 6 is a simplified diagram 600 of a cleaning and dispensing system
according to an embodiment of the present invention. This system 600 includes a
15 variety of elements such as a dispensing head 609, which is coupled to a plurality of
pipettes 601. The pipettes input and output fluids or solutions from plate 603. The
plate has a plurality of sites, each of which can be used to input cells or a combination
of cells and solution. The system also has elements to house solutions 605, which are
used to manipulate cell samples in the plate. The dispensing head is supported
20 through a support member 607, which is sufficiently rigid to allow for movement of
the head. The dispenser is coupled to the present system in a mechanical and
electrical manner, which provides for a fully integrated system for providing cell
samples to the imaging system according to the present invention.

Fig. 7A illustrates a representative block flow diagram of simplified
25 process steps of a method for determining properties of a manipulation based upon
effects of the manipulation on one or more portions of one or more cells in a
particular embodiment according to the present invention. This diagram is merely an
illustration and should not limit the scope of the claims herein. One of ordinary skill
in the art would recognize other variations, modifications, and alternatives. In step
30 700, one or more samples of cells can be provided. These cells can be live, dead, or
fixed cells, or cell fractions. The cells also can be in one of many cell cycle stages,
including G0, G1, S, G2 or M phase, M phase including the following cell cycle
stages: interphase, prophase, prometaphase, metaphase, anaphase, and telophase.

Cell components tracked in presently preferable embodiments can include proteins, protein modifications, genetically manipulated proteins, exogenous proteins, enzymatic activities, nucleic acids, lipids, carbohydrates, organic and inorganic ion concentrations, sub-cellular structures, organelles, plasma membrane, adhesion complex, ion channels, ion pumps, integral membrane proteins, cell surface receptors, G-protein coupled receptors, tyrosine kinase receptors, nuclear membrane receptors, ECM binding complexes, endocytotic machinery, exocytotic machinery, lysosomes, peroxisomes, vacuoles, mitochondria, Golgi apparatus, cytoskeletal filament network, endoplasmic reticulum, nuclear membrane, proteosome apparatus, chromatin, nucleolus, cytoplasm, cytoplasmic signaling apparatus, microbe specializations and plant specializations.

The following table illustrates some markers and cell components commonly used by embodiments according to the present invention. Other markers can be used in various embodiments without departing from the scope of the invention.

Cell component	Marker	Disease State
Plasma membrane (including overall cell shape)	Carbocyanine dyes Phosphatidylserine Various lipids Glycoproteins	Apoptosis-Cancer Apoptosis-Neural degenerative Ds
Adhesion complexes	Cadherins Integrins Occludin Gap junction ERM proteins CAMs Catenins Desmosomes	Thrombosis Metastasis Wound healing Inflammatory Ds Dermatologic Ds
Ion Channels and Pumps	Na/K Atpase Calcium channels Serotonin reuptake pump CFTR	Cystic fibrosis Depression Congestive Heart Failure Epilepsy

G coupled receptors	β adrenergic receptor Angiotensin receptor	Hypertension Heart Failure Angina
Tyrosine kinase receptors	PDGF receptor FGF receptor IGF receptor	Cancer Wound healing Angiogenesis Cerebrovascular Ds
ECM binding complexes	Dystroglycan Syndecan	Muscular Dystrophy
Endocytotic machinery	Clathrin Adaptor proteins COPs Presenilins Dynamin	Alzheimer's Ds
Exocytotic machinery	SNAREs Vesicles	Epilepsy Tetanus Systemic Inflammation Allergic Reactions
Lysosomes	Acid phosphatase Transferrin	Viral diseases
Peroxisomes/Vacuoles		Neural degenerative Ds
Mitochondria	Caspases Apoptosis inducing factor F1 ATPase Fluorescein Cyclo-oxygenase	Apoptosis Neural degenerative Ds Mitochondrial Cytopathies Inflammatory Ds
Golgi Apparatus	Lens Culinaris DiOC6 carbocyanine dye COPs	

Cytoskeletal Filament Networks	Microtubules Actin Intermediate Filaments Kinesin, dynein, myosin Microtubule associated proteins Actin binding proteins Rac/Rho Keratins	Cancer Neural degenerative Ds Inflammatory Ds Cardiovascular Ds Skin Ds
Endoplasmic Reticulum	SNARE PDI Ribosomes	Neural degenerative Ds
Nuclear Membrane	Lamins Nuclear Pore Complex	Cancer
Proteosome Apparatus	Ubiquityl transferases	Cancer
Chromatin	DNA Histone proteins Histone deacetylases Telomerases	Cancer Aging
Nucleolus	Phase markers	
Cytoplasm	Intermediary Metabolic Enzymes BRCA1	Cancer
Cytoplasmic Signaling Apparatus	Calcium Camp PKC pH	Cardiovascular Ds Migraine Apoptosis Cancer
Microbe Specializations	Flagella Cilia Cell Wall components: Chitin synthase	Infectious Ds

Plant specializations	Choloroplast Cell Wall components	Crop Protection
-----------------------	--------------------------------------	-----------------

Then, in a step 702, one or more samples of the manipulation can be provided to the cells. Manipulations can comprise one or any combination of chemical, biological, mechanical, thermal, electromagnetic, gravitational, nuclear, or temporal factors, for example. For example, manipulations could include exposure to chemical compounds, including compounds of known biological activity such as therapeutics or drugs, or also compounds of unknown biological activity. Or exposure to biologics that may or may not be used as drugs such as hormones, growth factors, antibodies, or extracellular matrix components. Or exposure to biologics such as infective materials such as viruses that may be naturally occurring viruses or viruses engineered to express exogenous genes at various levels. Bioengineered viruses are one example of manipulations via gene transfer. Other means of gene transfer are well known in the art and include but are not limited to electroporation, calcium phosphate precipitation, and lipid-based transfection. Manipulations could also include delivery of antisense polynucleotides by similar means as gene transfection. Other genetic manipulations include gene knock-outs or gene mutations. Manipulations also could include cell fusion. Physical manipulations could include exposing cells to shear stress under different rates of fluid flow, exposure of cells to different temperatures, exposure of cells to vacuum or positive pressure, or exposure of cells to sonication. Manipulations could also include applying centrifugal force. Manipulations could also include changes in gravitational force, including sub-gravitation (the preferred embodiment in outer space). Manipulations could include application of a constant or pulsed electrical current. Manipulations could also include irradiation. Manipulations could also include photobleaching which in some embodiments may include prior addition of a substance that would specifically mark areas to be photobleached by subsequent light exposure. In addition, these types of manipulations may be varied as to time of exposure, or cells could be subjected to multiple manipulations in various combinations and orders of addition. Of course, the type of manipulation used depends upon the application.

Then, in a step 704, one or more descriptors of a state in the portions of the cells in the presence of the manipulation can be determined using the images

collected on the imaging system. Descriptors can comprise scalar or vector values, representing quantities such as area, perimeter, dimensions, intensity, gray level, aspect ratios, and the like. Other types of descriptors include, but are not limited to, one or any combination of characteristics such as a cell count, an area, a perimeter, a length, a breadth, a fiber length, a fiber breadth, a shape factor, a elliptical form factor, an inner radius, an outer radius, a mean radius, an equivalent radius, an equivalent sphere volume, an equivalent prolate volume, an equivalent oblate volume, an equivalent sphere surface area, an average intensity, a total intensity, and an optical density. These descriptors can be average or standard deviation values, or frequency statistics from the descriptors collected across a population of cells. These descriptors can be further reduced using other methods such as principal component analysis and the like. In some embodiments, the descriptors include features from different cell portions or cell types. That is, a first feature can be from a nuclei and a second feature is from another cell structure such as Golgi apparatus, mitochondria, spacing between cell structures or cells themselves, as well as many others.

A presently preferable embodiment uses descriptors selected from the following table. Other descriptors can also be used without departing from the scope of the invention.

Name of Parameter	Explanation/Comments
Count	Number of objects
Area	
Perimeter	
Length	X axis
Width	Y axis
Shape Factor	Measure of roundness of an object
Height	Z axis
Radius	
Distribution of Brightness	
Radius of Dispersion	Measure of how dispersed the marker is from its centroid
Centroid location	x-y position of center of mass
Number of holes in closed objects	Derivatives of this measurement might include, for

	example, Euler number (= number of objects - number of holes)
Elliptical Fourier Analysis (EFA)	Multiple frequencies that describe the shape of a closed object
Wavelet Analysis	As in EFA, but using wavelet transform
Interobject Orientation	Polar Coordinate analysis of relative location
Distribution Interobject Distances	Including statistical characteristics
Spectral Output	Measures the wavelength spectrum of the reporter dye. Includes FRET
Optical density	Absorbance of light
Phase density	Phase shifting of light
Reflection interference	Measure of the distance of the cell membrane from the surface of the substrate
1,2 and 3 dimensional Fourier Analysis	Spatial frequency analysis of non closed objects
1,2 and 3 dimensional Wavelet Analysis	Spatial frequency analysis of non closed objects
Eccentricity	The eccentricity of the ellipse that has the same second moments as the region. A measure of object elongation.
Long axis/Short Axis Length	Another measure of object elongation.
Convex perimeter	Perimeter of the smallest convex polygon surrounding an object
Convex area	Area of the smallest convex polygon surrounding an object
Solidity	Ratio of polygon bounding box area to object area.
Extent	proportion of pixels in the bounding box that are also in the region
Granularity	
Pattern matching	Significance of similarity to reference pattern
Volume measurements	As above, but adding a z axis

Then, in a step 705, a database of cell information can be provided. Next, in a step 706, a plurality of descriptors can be searched from a database of cell information in order to locate descriptors based upon one of the descriptors of the manipulation. Then, in a step 708, properties of the manipulation are predicted based
5 upon the properties of the located descriptors. Properties can comprise toxicity, specificity against a subset of tumors, mechanisms of chemical activity, mechanisms of biological activity, structure, adverse biological effects, biological pathways, clinical effects, cellular availability, pharmacological availability, pharmacodynamic properties, clinical uses and indications, pharmacological properties, such as
10 absorption, excretion, distribution, metabolism and the like.

In a particular embodiment, step 706 comprises determining matching descriptors in the database corresponding to a prior administration of the manipulation to the descriptors of the present administration of the manipulation. In a particular embodiment according to the present invention, combinations of
15 measurements of scalar values can provide predictive information. A database can be provided having one or more "cellular fingerprints" comprised of descriptors of cell-substance interactions of drugs having known mechanisms of action with cells. Such descriptors can be analyzed, classified, and compared using a plurality of techniques, such as statistical classification and clustering, heuristic classification techniques, a
20 technique of creating "phylogenetic trees" based on various distance measures between descriptors from various drugs. In this embodiment, numeric values for the descriptors can be used by comparison techniques. A phylogenetic tree can be created that illustrates a statistical significance of the similarity between descriptors for the drugs in the database. Because the drugs used to build the initial database are of
25 known mechanism, it can be determined whether a particular scalar value in a descriptor is statistically predictive. Finally, a compound descriptor with no known mechanism of action can be queried against the database and be statistically compared and classified among the drugs in the database that the compound most resembles.

In a particular embodiment, relationships between measured
30 morphological properties of images and physiological conditions can be determined. Relationships can include, for example, treatment of different cell lines with chemical compounds, or comparing cells from a patient with control cells, and the like. In a presently preferable embodiment, comparisons can be performed on acquired image

features. Some embodiments can comprise statistical and neural network - based approaches to perform comparisons of various features. The foregoing is provided as merely an example, and is not intended to limit the scope of the present invention. Other techniques can be included for different types of data.

5 In some embodiments, classification, clustering and other types of predictive data analysis can be performed on features extracted from cell images. In a presently preferable embodiment, statistical procedures for comparisons, classification and clustering are performed on data obtained from imaging cells.

Fragments of data preparation and pre-formatting (S language):

```
10       >tmp.frame <- Generic.Summary  
      >names1 <- paste("Cell.line.5", tmp.names, sep=".")  
      > by.compound.matrix <- as.matrix(arranged.by.compound)
```

 Example of the code for principal component analysis (data
15 preparation) using S language:

```
      all.data.princomp <- menuPrincomp(data =  
      by.compound.matrix, scores = T, cor = "Correlation",  
      na.action = T, print.short = T, print.importance = T,  
      print.loadings = T, cutoff.loadings = 0.1,  
20       plot.screplot = T, plot.loadings = T, plot.biplot = T,  
      plot.biplot.choices = c(1,2), predict.p = F)
```

 Example of clustering using a divisive hierarchical clustering
algorithm:

```
25       > div.hier.2.manhattan.cluster$call  
      diana(x = tmp.sum.by.comp, diss = F, metric =  
      "manhattan",  
          stand = T, save.x = T, save.diss = T)
```

30 Another embodiment utilizes existing tools for biological sequence similarity searches, classification, and phylogenetic analysis. In a particular embodiment, numbers in a numerical descriptor can be substituted by one or more of nucleic acid or amino acid codes according to a one of several sets of rules. Once

converted into a corresponding nucleotide or amino acid sequence representation, the fingerprints can be analyzed and compared using software and algorithms known in the art for genetic and peptide sequence comparisons, such as GCG, a product of Genetics Computer Group, with company headquarters in Madison WI. Select
5 embodiments comprising such approaches enable the use of a broad array of sophisticated algorithms to compare, analyze, and cluster gene and protein sequences. Many programs performing this task are known to those of ordinary skill in the art, such as for example, the PHYLIP (PHYlogeny Interference Package) a package of programs for inferring phylogenies (evolutionary trees) described in (Feldenstein, J.
10 1996 Methods Enzymol 266:418-427 and Feldenstein, J. 1981 J. Mol. Evol. 17(6):368-376).

Embodiments can perform such analysis based upon factors such as numerical value, statistical properties, relationships with other values, and the like. Further details of a step of manipulation are noted more particular below.

15 Fig. 7B illustrates a representative block flow diagram of simplified process steps for determining one or more descriptors of a state in the portions of the cells in the presence of the manipulation of step 704 of Fig. 7A in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill
20 in the art would recognize other variations, modifications, and alternatives. In a step 712, an image of a cell portion is obtained. In some embodiments, the cell portion is visualized with a fluorescently labeled marker that is specific for the portion or portions of interest. A cell portion can include, for example, one or more of the following: nuclei, Golgi apparatus, and other features. The cell portion may vary in
25 select embodiments according to the invention. Then, in a step 714, a digitized representation of the image obtained in step 712 is determined. In some embodiments, steps 714 and step 712 can comprise a single step. These embodiments use a digital imaging means such as a digital camera, to obtain a digital image of the target directly. Next, in a step 716, the digital representation of the image is
30 processed to obtain image features. Image features can include such quantities as area, perimeter, dimensions, intensity, aspect ratios, and the like. Then, in a step 718 descriptors can be determined from the image features. Descriptors can comprise scalar or vector quantities and can comprise the image features themselves, as well as

composed features, such as shape factor derived by a relationship $4\pi * \text{area} / \text{perimeter}$, and the like. Descriptors can also comprise statistical quantities relating to feature characteristics across a population of cells, such as a standard deviation, and average, and the like.

5 In a preferred embodiment, cells can be placed onto a microscope, such as a Zeiss microscope, or its equivalent as known in the art. A starting point, named Site A01, is identified to the microscope. A plurality of exposure parameters can be optimized for automated image collection and analysis. The microscope can automatically move to a new well, automatically focus, collect one or more images, at
10 one or more wavelengths, move to a next well, and repeat this process for all designated wells in a multiple well plate and for multiple plates. A file having a size and an intensity distribution measurement for each color and rank for each well can then be created for the images acquired. Based on this information, a user or a computer can revisit sites of interest to collect more data, if desired, or to verify
15 automated analysis. In a presently preferred embodiment, image automatic focus and acquisition can be done using computer software controlling the internal Z-motor of the microscope. Images are taken using a 10x, 20x, or 40x air long working distance objectives. Sometimes multiple images are collected per well. Image exposure times can be optimized for each fluorescent marker and cell line. The same exposure time
20 can be used for each cell line and fluorescent marker to acquire data.

Fig. 7C illustrates a representative block flow diagram of simplified process steps for obtaining images of cell portions of step 712 of Fig. 7B in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill
25 in the art would recognize other variations, modifications, and alternatives. The method is generally outlined by the steps below:

(1). In a step 720, a sample is provided to the imaging device. Samples can be provided in 96 well plates and the like. The sample may be loaded into a microscope, such as a Zeiss microscope or equivalent.

30 (2). In a step 722, a set of optical filters is selected to shine light of the appropriate wavelength to illuminate the first sample, which may be contained in a first well designated A01.

(3). In a step 724, an automatic focusing procedure is performed for the site. In a particular embodiment, the internal z-motor of the microscope which is attached to the objective nosepiece is used for automatic focusing of the microscope. In an alternative embodiments, the plate holding the samples is moved to perform automatic focusing of the microscope, or focusing can be performed by moving optical components attached to the microscope and the like.

(4). In a step 726, images are collected for the site. Images can be collected for every color at every site. Present embodiments can provide images for up to four colors. However, embodiments are contemplated that can provide more colors by using either a monochromator coupled with excitation filters which are on a filter wheel, or by digitally separating overlapping fluorophores. Those knowledgeable in the field will know that given calibration images of single fluorophores, a look-up table can be devised which will allow for the digital removal of fluorescence bleed-through of fluorescence which may occur in optical channels other than the one for which that filter has been optimized in instances of using more than one fluorophore at once. Cell growth and density information is also collected. Cell density is determined by what percentage of the area being imaged is inhabited by cells. In some embodiments, imaging can be facilitated using one or more biosensors, molecules such as non-proteins, i.e., lipids and the like, that are luminescently tagged. However, some embodiments can also use fluorescence polarization and the like. Fluorescence polarization is a homogeneous fluorescence technology where the excited state of the molecule lasts much longer than in normal fluorescence, taking seconds to minutes to reach equilibrium, obliterating the need to wash away fluorescence markers that are not specifically bound to a marker. Further, embodiments can detect differences in spectral shifts of luminescent markers. Some fluorescence markers, such as Nile Red sold by Molecular Probes of Eugene, OR, will change its emission peak wavelength depending on its environment. One can detect these changes by monitoring the level of fluorescence at both wavelengths and reading out at ratio of the two.

(5). In a step 728, a determination is made whether more fields of view need to be taken for a particular color. If this is so, then processing continues at step 726 at a new site. Otherwise, processing continues with a decisional step 730.

Images can now be taken by repeating step 726. In a preferred embodiment 4 to 9 images are collected at each site.

(5). In a step 730, a determination is made whether more optical configurations need to be taken in order to obtain images for all differently-marked cell portions the sample. If this is so, then in a step 732 a new optical configuration is determined. Images for the new optical configuration can now be taken by repeating steps 726 and 728.

(6). In a decisional step 734, after all optical configurations and images for fields of view in a sample have been obtained, a determination is made whether any further samples remain to be analyzed. If so, a new sample is brought into view and processing continues with step 720. Otherwise, image processing is complete. In a presently preferable embodiment, image data can be stored on a CD ROM using a CD ROM burner, such as CRW4416 made by Yamaha of Japan. However, other mass storage media can also be used.

Fig. 7D illustrates a representative block flow diagram of simplified process steps for processing digitized representations of step 716 of Fig. 7B in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. The method is generally outlined by the steps below:

(1). In a step 740, a digitized image input is preprocessed .

Preprocessing might include, but is not limited to, such operations as background subtraction, thresholding, smoothing, adoptive filtering, edge enhancements, contrast enhancements, histogram equalization. A particular combination of preprocessing steps can be applied to images in successive steps or in parallel to copies of the image.

A simplified example of a smoothing and background subtraction procedure in a MatLab language is presented in computer code below:

```
function Isubtracted = cmBackgrSubtrl(I,k)
```

```
% cmBackgrSubtrl(I,k) - simple flat background (=modal*k)  
subtraction
```

```
% Y = cmBackgrSubtrl(I, k) - image Y is generated by
```

```

    % subtraction (with saturation) of modal pixel value of I
    multiplied by k
    % DEFAULT - k=1
    %
5   if (nargin == 1)
        k=1;
    end
    if (size(k)~=1)
        error('cmBackgrSubtrl: parameter k should be a number.
10  Exiting...');
    end

    %modpixnum = floor(size(I(:),1)/2);
    %sortedval = sort( double(I(:)) );
15  %modpixel = sortedval(modpixnum);
    modpixel = median(double(I(:)));
    bg = k*modpixel;

    Isubtracted = mmsubm( uint8(I), uint8(round(ones(
20  size(I))*k*modpixel )) );

```

An example of a procedure for thresholding in computer code (MatLab) is presented below:

```

function thresh = GetThreshByPerim1(I, M)
25  % GetThreshByPerim1(I) Finds optimal thresholding value
    for image I
    % N = GetThreshByPerim1(I) Finds thresholding value N for
    image I
    % N = GetThreshByPerim1(I, M) - tests threshold values up
30  to M
    % DEFAULT M = maximum pixel value in I
    % note that GetThreshByArea is significantly faster
    % finds a threshold value that causes the maximal change
    in the

```

```
% total perimeter of the objects (Russ ????)
% see Matlab_Auto_threshold1_1-23-99.doc for more details
% Note: works somewhat better on SMOOTH images (i.e.
medfilt2(I, [3 3]) two times

5
if (nargin == 0)
    error (strcat( mfilename, ' : at least one parameter
required'));
elseif (nargin == 1)
10    M = double(max(I(:)));      %test thresholds up to
maximum pixel value in I
elseif (nargin > 2)
    error (strcat (mfilename, ' : too many parameters'));
end

15
if (size(M)>1)
    error (strcat(mfilename, ' : argument M should be a
number'));
end

20
Minval = double( min(I(:)));
step = 1;

%generate vertical vector perims with total perimeters of
25 objects at different
%threshold values
for i=Minval : step : M
    bwI = im2bw(I, i/255);
    prI = bwperim(bwI);
30    pr = sum(prI(:));
    if (exist('perims', 'var') == 0) %perims is yet
undefined
        perims = pr;
    else
```



```

        perims = cat(1, perims, pr);
    end
end

5  % vector prdiffs contains differences between successive
   perimeters
   prdiffs = diff(perims);
   mindecrease = min(prdiffs);
   minvalues = find(prdiffs == mindecrease);
10  index_of_mindecrease = minvalues(1);
   thresh = index_of_mindecrease + 1;

   % =====end GetThresh1=====

```

15 Thresholding provides a specific intensity, such that pixels darker than the threshold are deemed black, and pixels lighter than the threshold are considered white. The thresholded image can be processed using binary image processing techniques in order to extract regions.

(2). In a step 742+744, the digitized image input is subjected to object
20 identification. This can be accomplished by a variety of procedures, for example by thresholding or edge detection and subsequent morphological opening and closing. Edge detection can be accomplished by means of gradient-based or zero-crossing methods, such as Sobel, Canny, Laplassian, Perwitt, and other methods.

An example of object identification procedure based on Canny edge
25 detection (in MatLab language) is presented below:

```

function Imask = cmMaskDNA1(I);
% cmMaskDNA1 - generates binary mask for cell nuclei
% through edge detection
30 % Imask = cmMaskDNA1(I)
% PARAMETERS
%   I - intensity image (grayscale)
% OUTPUT
%   Imask - BW image with objects from I

```

```

%
% For more details see Notebook Matlab_DNA_masking1_1-22-
99.doc
% Uses SDC Morphology Toolbox V0.7
5
if (nargin ~= 1)
    error('Wrong number of input parameters');
end
if (nargout ~= 1)
10    error('Wrong number of output parameters: one output
argument should be provided');
end

15  Imask = edge(I, 'canny');
    Imask = mm dil(Imask, mmsecross(1));
    Imask = mmero ( mmc lohole(Imask, mmsecross(1)) );
    Imask = mmedgeoff(Imask, mmsecross(1));
    % note that mmedgeoff this command removed FILLED OBJECTS
20  but not touching OUTLINES.
    % these outlines can be removed by filtering:
    Imask = medfilt2(Imask, [5 5]);

    %=====end cmMaskDNA1
25  =====

```

However, embodiments can also use other techniques, such as Fast Fourier Transforms (FFT) and the like as known in the art without departing from the scope of the present invention.

30 (3). In a step 746, a plurality of region features can be determined. For example, in a representative embodiment, image features can include such quantities as area, perimeter, dimensions, intensity, aspect ratios, and the like. Features not directly related to individual objects are also being extracted.

An example of a procedure for extraction of some of the features (MatLab language) is presented below:

```

function OData = cmGetObjectsData(I, Ilabel)
5  % cmGetObjectsData returns array measurements of objects
  in image "I" masked by "Ilabel"
  % EV 2-3-99; 2-10-99
  % OData = cmGetObjectsData(I, Ilabel) returns an array of
  morphological and intensity measurements
10  %   taken from a grayscale image "I". Objects are
  identified on a mask image Ilabel, usually
  %   created by bwlabel()
  % OUTPUT:
  % Each row in the output array OData represents
15  individual object
  % columns contain the following measurements:
  %
  %   1 - Index ("number" of an object);      8 -
  Solidity;
20  %   2 - X coordinate of the center of mass; 9 - Extent;
  %   3 - Y coordinate      "-"      ; 10 - Total
  Intensity;
  %   4 - Total Area (in pixels);              11 - Avg.
  Intensity;
25  %   5 - Ratio of MajorAxis/MinorAxis;      12 - Median
  Intensity;
  %   6 - Eccentricity;                        13 - Intensity of
  20% bright pixel
  %   7 - EquivDiameter;                       14 - Intensity of
30  80% bright pixel
  %
  % For details on morphological parameters see information
  on MatLab imfeature();

```

```
% Intensity parameters are either obvious or are
documented in comments in this file.

if (nargin ~= 2)
5   error ('function requires exactly 2 parameters');
end
if (nargout ~= 1)
    error ('function has 1 output argument (array X by
14)');
10 end

% finished checking arguments

% first collect morphological parameters in a structure
15 array:
ImStats = imfeature(Ilabel, 'Area', 'Centroid',
    'MajorAxisLength',...
    'MinorAxisLength', 'Eccentricity', 'EquivDiameter',
    ...
20 'Solidity', 'Extent', 8 );

% now convert it into array (matrix) while collecting
intensity data for each object:

25 %preallocate output array:
numobjects = size(ImStats, 1);
OData = zeros(numobjects, 14);
%now convert ImStats into array and add intensity data to
it
30 for k=1:numobjects
    OData(k, 1) = k;
    OData(k, 2) = ImStats(k).Centroid(1);
    OData(k, 3) = ImStats(k).Centroid(2);
    OData(k, 4) = ImStats(k).Area;
```

```

        OData(k, 5) = (ImStats(k).MajorAxisLength) /
        (ImStats(k).MinorAxisLength);
        OData(k, 6) = ImStats(k).Eccentricity ;
        OData(k, 7) = ImStats(k).EquivDiameter;
5       OData(k, 8) = ImStats(k).Solidity;
        OData(k, 9) = ImStats(k).Extent;

        % now collect and assign intensity parameters from
        image I
10
        object_pixels = find( Ilabel == k);
        object_area = size(object_pixels, 1); %same as total
        number of pixels in the object
        object_intensities = double(I(object_pixels)); %
15    need to convert to double to do math
        sorted_intensities = sort(object_intensities); %
        will need to get median, 20% and 80% pixels
        total_intensity = sum(object_intensities, 1);
        avg_intensity = total_intensity / object_area;
20    median_intensity = sorted_intensities( floor(
        object_area/2 ) + 1 );
        pix20 = sorted_intensities( floor(object_area*0.2)+1
        ) ; %brightest pixel among dimmest 20%
        pix80 = sorted_intensities( floor(object_area*0.8)+1
25    ) ;

        OData(k, 10) = total_intensity;
        OData(k, 11) = avg_intensity;
        OData(k, 12) = median_intensity;
30    OData(k, 13) = pix20; %brightest pixel among dimmest
        20%
        OData(k, 14) = pix80; %dimmest pixel among brightest
        20%
        end %for

```

```
%===== end function
cmGetObjectsData() =====
```

- 5 (4). In a step 748, quantitative descriptors, characterizing cell state are calculated based on the feature measurements extracted at step 746. For example, histogram distribution of intensities of cell nuclei provides information about the population cell cycle stages.

In a particular embodiment according to the present invention, data analysis techniques for describing the fluorescence patterns of cell portions in multiple cell lines in the presence and absence of compounds are provided. Automated image analysis techniques can include determining one or more regions from around nuclei, individual cells, organelles, and the like, called "objects" using a thresholding function. Objects that reside on the edge of an image can be included or excluded in various embodiments. An average population information about an object can be determined and recorded into a database, which can comprise a database text file or Excel spreadsheet, for example. However, embodiments can use any recording means without departing from the scope of the present invention. Values measured can be compared to the visual image. One or more types of numerical descriptors can be generated from the values. For example, descriptors such as a number of objects, an average, a standard deviation of objects, a histogram (number or percentage of objects per bin, average, standard deviation), and the like can be determined.

In a particular embodiment according to the present invention, data can be analyzed using morphometric values derived from any of a plurality of techniques commonly known in the art. For example, a software package called MetaMorph Imaging System, provided by Universal Imaging Corporation, a company with headquarters in West Chester, PA and NIH Image, provided by Scion Corporation, a company with headquarters in Frederick, Maryland.

30 Fluorescent images can be described by numerical values, such as for example, an area, a fluorescence intensity, a population count, a radial dispersion, a perimeter, a length, and the like. Further, other values can be derived from such measurements. For example, a shape factor can be derived according to a relationship

4π * area / perimeter. Other values can be used in various embodiments according to the present invention. Such values can be analyzed as average values and frequency distributions from a population of individual cells.

In a particular embodiment according to the present invention, techniques for the automatic identification of mitotic cells are provided. Image analysis techniques employing techniques such as multidimensional representations, frequency-based representations, multidimensional cluster analysis techniques and the like can be included in various embodiments without departing from the scope of the present invention. Techniques for performing such analyses are known in the art and include those embodied in MatLab software, produced by MathWorks, a company with headquarters in Natick, MA.

Scalar values providing efficacious descriptors of cell images can be identified using the techniques of the present invention to perform predictive analysis of drug behavior. In a presently preferred embodiment, a plurality of heterogeneous scalar values can be combined to provide descriptors for each manipulation. By applying predictive analysis routines to the collections of these descriptors, predictive information about any number of manipulations and cell interactions can be extracted.

Fig. 7E illustrates a representative block flow diagram of simplified process steps for analyzing image feature values to obtain descriptors of cell state of step 718 of Fig. 7B in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. Fig. 7E illustrates an input data of descriptors of known manipulations 319. A step 320 of reformatting and transforming data 319 to formats suitable for analysis is performed. Additionally, a "cleaning" process can eliminate outlying data points and the like in the data. Then, in a step 322, a decision is made whether to continue with step 324 or with step 326 based upon determining a particular type of analysis appropriate for the present application or particular type of prediction. If decisional step 322 determines processing should continue with step 324, then, in that step, an error estimate using a set of test descriptors is performed to estimate the quality of a prediction and processing continues with step 320. Once an optimal prediction is achieved, processing continues with step 326. In step 326, optimal transformation parameters and prediction methods are selected for use in

steps 328 and 330 which analyze data about an unknown manipulation. In a step 328, a solution is generated based upon any of techniques including training a neural network, solving a mathematical equation, applying decision tree rules and/or the like. In a step 330, an input data set of unknown descriptors 318 is reformatted and
5 transformed based upon the optimal transformation parameters selected in step 326 using the transformation procedures in steps 320, 322 and 324. In a step 332, predictions techniques are applied to the reformatted manipulations from step 330 and the solution generated in step 328 and a plurality of properties of known manipulations 317 (e.g., therapeutic properties, and the like) in order to determine a
10 prediction of properties of unknown manipulation 316.

Fig. 7F illustrates a representative block flow diagram of simplified process steps for a method of mapping a manipulation of cells to a physiological characteristic in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein.
15 One of ordinary skill in the art would recognize other variations, modifications, and alternatives. The method is generally outlined by the steps below:

(1) In a step 750, a plurality of cells, e.g., dead, live, cell fractions or mixtures of cells are provided.

(2) Then, in a step 752, the plurality of cells is manipulated, where
20 manipulation occurs using a source(s) from one or a combination selected from an electromagnetic, electrical, chemical, thermal, gravitational, nuclear, temporal, or a biological source.

(3) Next, in a step 754, a feature value is captured from the plurality of cells. The feature value can include one or any combination of characteristics such as
25 cell count, area, perimeter, length, breadth, fiber length, fiber breadth, shape factor, elliptical form factor, inner radius, outer radius, mean radius, equivalent radius, equivalent sphere volume, equivalent prolate volume, equivalent oblate volume, equivalent sphere surface area, average intensity, total intensity, and optical density. This list is not meant to be limiting.

(4) Then, in a step 756, a degree of presence of one or more feature values is assigned for each manipulation.
30

(5) In a step 758, the feature values from the plurality of cells are stored in memory locations. From the memory locations the values can be used for

statistical analyses to produce predictive information about the relatedness of the descriptors of the manipulations to one another. This information is used to infer properties of the manipulations.

Fig. 7G illustrates a representative block flow diagram of a simplified process steps for a method for populating a database with manipulated biological cell information in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. The method is generally outlined by the steps below:

10 (1) In a step 760, a plurality of cells in various stages of the cell cycle, A montage image that was used as a source to generate data in Appendix A is presented in Fig. 12., such as for example, the stages of interphase, prophase, metaphase, anaphase, and telophase are provided.

(2) Then, in a step 762, each of the cells in the various stages of mitotic development is manipulated.

(3) Next, in a step 764, an image of the plurality of manipulated cells is captured using image acquisition techniques in order to provide a morphometric characteristic of each of the manipulated cells.

(4) As a preferable option, in a step 766, an image database may be populated with the image of the plurality of manipulated cells.

(5) Following step 764 or optional step 766, a morphological value is calculated from the image in a step 768.

(6) In a step 770, the database is populated with the morphological value.

25 Fig. 7H illustrates a representative block flow diagram of simplified process steps for a method for populating a database with manipulated biological information, e.g., image acquisition parameters, image feature summary information, and well experimental parameters in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. Fig. 7H illustrates a step 780 in which cells are placed into site on a plate and a manipulation is applied. Then, in a step 781 an image is taken of the cells. In step 782, the image is transferred to an image archive

database. Then, in a step 783, well experimental parameters are entered into the database 787. Well experimental parameters can include cell type, manipulation and the like. In a step 784, image acquisition parameters are transferred to database 787. Image acquisition parameters can include file name, fluorophores and the like. In a
5 step 785, the image acquired in step 781 is analyzed. Then, in step 786, an image feature summary from the analysis step 785 is transferred to database 787.

In step 788, a lookup table for all analyses is provided to database 787. The lookup table provides information about the analyses. In a step 789, a query of database 787 for process data is performed. The results are reformatted. Then in a
10 step 790, the database 787 is queried. Next, in a step 791, features of the manipulations stored in the database are combined and reduced. Next, in a step 793, reduced features of step 791 can be compared. In a step 792, the results of step 793 are recorded in database 787. Then, in a step 794, a report of predictions based on comparisons performed in step 793 is generated.

15 Fig. 7I illustrates a representative block flow diagram of simplified process steps for acquiring images of manipulated biological information, e.g., cells, cell tissues, and cell substituents in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations,
20 modifications, and alternatives. Fig. 7I illustrates a step 770 in which a user sets up an image analysis procedure. Then, in a step 772, an image is read into image analysis software. Next, in a step 774, patterns and objects are identified in the image using one or more algorithms. Next, in a step 776, sets of features are extracted from the image. Then, in a step 778, feature information, descriptor values and the like are
25 exported to the database, such as database 787 of Fig. 7H, for recording. Next, in a decisional step 779, a determination is made whether any more images should be taken. If this is so, processing continues with step 772. Otherwise, image acquisition processing is completed.

Fig. 7J illustrates a representative block flow diagram of simplified
30 process steps for populating, acquiring and analyzing images of manipulated biological information in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations,

modifications, and alternatives. Fig. 7J illustrates a step 300 of placing a plate onto an imaging stage and reading a bar code. Then, in a step 301 an autofocus procedure is performed. Next, in a step 302, a first optical filter configuration is selected and an image is collected. Then, in a decisional step 303, a determination is made whether
5 more than one image per optical configuration can be taken. If so, then, in a step 304, a new position within the well is targeted and another image is collected. Then, in a decisional step 305, a determination is made whether any more images need to be collected. If this is so, step 304 is repeated until all images for a particular well have been collected. After one or more images are collected for the well, in a step 306, the
10 stage is returned to a starting position within the well, and a montage is created from collected images. The results are named with a unique file name and stored.

In a decisional step 307, a determination is made whether any more optical channels in the well can be imaged. If this is so, then in a step 308 the next optical filter configuration is selected and an image is collected. Processing then
15 continues with decisional step 303, as described above. Otherwise, if no further optical channels in the well can be imaged, then in a decisional step 309 a determination is made whether any wells remain to be imaged. If not all wells have been imaged, then in a step 310, the stage moves to the next well and processing continues with step 301, as described above. Otherwise, if all wells on the plate have
20 been imaged, then in a decisional step 311, a determination is made whether any more plates can be processed. If this is so, then processing continues with step 300 as described above. Otherwise, in a step 312, the information is stored to a CD or other storage device as a backup.

Fig. 7K illustrates a representative block flow diagram of simplified
25 process steps compound based upon information about effects of one or more known compounds on a cell population in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. Fig. 7K illustrates a step 340 of populating a database
30 with descriptors for known compounds. Such descriptors can be determined from imaging the cell population. However, in some embodiments, descriptors can be derived by measurements and combinations of measurements and the like. Then, in a step 342, descriptors for the unknown compound are determined from imaging a

second cell population. The second cell population has been treated with the unknown compound. Then, in a step 344, a relationship between the descriptors determined from the unknown compound with the descriptors determined from the known compounds can be determined. Finally, in a step 346, an inference can be made about the unknown compound based upon the descriptors of the known compounds from the relationship determined in step 344.

Accordingly, the present invention provides a novel database design. In a particular embodiment according to the present invention, a method for providing a database comprises measurement of a potentially large number of features of one or more sub-cellular morphometric markers. Markers can be from any of a large variety of normal and transformed cell lines from sources such as for example, human beings, fungi, or other species. The markers can be chosen to cover many areas of cell biology, such as, for example markers comprising the cytoskeleton of a cell. The cytoskeleton is one of a plurality of components that determine a cell's architecture, or "cytoarchitecture". A cytoarchitecture comprises structures that can mediate most cellular processes, such as cell growth and division, for example. Because the cytoskeleton is a dynamic structure, it provides a constant indication of the processes occurring within the cell. The cytoarchitecture of a cell can be quantified to produce a one or more scalar values corresponding to many possible cellular markers, such as cytoskeleton, organelles, signaling molecules, adhesion molecules and the like. Such quantification can be performed in the presence and absence of drugs, peptides, proteins, anti-sense oligonucleotides, antibodies, genetic alterations and the like. Scalar values obtained from such quantification can provide information about the shape and metabolic state of the cell.

In a presently preferred embodiment, scalar values can comprise morphometric, frequency, multi-dimensional parameters and the like, extracted from one or more fluorescence images taken from a number of cellular markers from a population of cells. Two or more such scalar values extracted from a plurality of cell lines and markers grown in the same condition together comprise a unique "fingerprint" or descriptor that can be incorporated into a database. Such cellular descriptors will change in the presence of drugs, peptides, proteins, antisense oligonucleotides, antibodies or genetic alterations. Such changes can be sufficiently unique to permit a correlation to be drawn between similar descriptors. Such

correlations can predict similar properties or characteristics with regard to mechanism of action, toxicity, animal model effectiveness, clinical trial effectiveness, patient responses and the like. In a presently preferred embodiment, a database can be built from a plurality of such descriptors from different cell lines, cellular markers, and
5 compounds having known mechanisms of action (or structure, or gene response, or toxicity).

The present invention also provides database and descriptor comparisons according to other embodiments. In a particular embodiment according to the present invention, measurement of scalar values or features can provide
10 predictive information. A database can be provided having one or more "cellular fingerprints" comprised of descriptors of cell substance interactions of drugs having known mechanisms of action with cells. Such descriptors can be compared using a plurality of techniques, such as a technique of creating "phylogenetic trees" of a statistical similarity between the descriptors from various drugs. In a present
15 embodiment, scalar, numeric values can be converted into a nucleotide or amino acid letter. Once converted into a corresponding nucleotide representation, the descriptors can be analyzed and compared using software and algorithms known in the art for genetic and peptide sequence comparisons, such as GCG, a product of Genetics Computer Group, with company headquarters in Madison WI. In an alternative
20 embodiment, numeric values for the fingerprints can be used by comparison techniques. A phylogenetic tree can be created that illustrates a statistical significance of the similarity between descriptors for the drugs in the database. Because the drugs used to build the initial database are of known mechanism, it can be determined whether a particular scalar value in a descriptor is statistically predictive. Finally, a
25 compound fingerprint with no known mechanism of action can be queried against the database and be statistically compared and classified among the drugs in the database that the compound most resembles.

In a particular embodiment, relationships between measured morphometric properties and features of images and physiological conditions can be
30 determined. Relationships can include, for example, treatment of different cell lines with chemical compounds, or comparing cells from a patient with control cells, and the like. In a presently preferable embodiment, a clustering can be performed on acquired image descriptors. Some embodiments can comprise statistical and neural

network - based approaches to perform clustering and comparisons of various descriptors. The foregoing is provided as merely an example, and is not intended to limit the scope of the present invention. Other techniques can be included for different types of data. In some embodiments, clustering and comparing can be performed on features extracted from cell images. In a presently preferable embodiment, procedures for comparisons and phylogenetic analysis of biological sequences can be applied to data obtained from imaging cells.

Select embodiments comprising such approaches enable the use of a broad array of sophisticated algorithms to compare, analyze, and cluster gene and protein sequences. Many programs performing this task are known to those of ordinary skill in the art, such as for example, the program Phylip, available at <http://evolution.genetics.washington.edu/phylip.html>, and other packages listed at <http://evolution.genetics.washington.edu/phylip/software.html>. However, select embodiments according to the present invention can comprise a technique of statistical classification, statistical clustering, distance based clustering, linear and non-linear regression analysis, self-organizing networks, and rule-based classification.

Embodiments can perform such analysis based upon factors such as numerical value, statistical properties, relationships with other values, and the like. In a particular embodiment, numbers in a numerical descriptor can be substituted by one or more of nucleic acid or amino acid codes. Resulting "pseudo-sequences" can be subjected to analysis by a sequence comparison and clustering program.

Other types of databases can also be provided according to other embodiments. The database includes details about the properties of a plurality of standard drugs. When the descriptor of a test compound is compared to the database, predictions about the properties of the test compound can be made using any known property of the other compounds in the database. For example, properties about a compound in the database could include structure, mechanism of action, clinical side effects, toxicity, specificity, gene expression, affinity, pharmacokinetics, and the like. The descriptor of a compound of unknown structure from a natural products library could be compared to the descriptors of compounds with known structure and the structure could be deduced from such a comparison. Similarly, such information could lead to better approaches to drug discovery research including target validation

and compound analogizing, as well as pre-clinical animal modeling, clinical trial design, side effects, dose escalation, patient population and the like.

According to the present invention, databases can be integrated with and complementary to existing genomic databases. Differential genomic expression strategies can be used for drug discovery using database technology. In one particular embodiment, cell data and cellular response data can be associated with a genetic expression profile assay to form a single assay. Live cells expressing fluorescence markers can be treated with a drug, imaged and analyzed for morphometry; and then analyzed for mRNA for expression. Such embodiments can provide rapid development of tools to link cellular behavior with functional genomics.

Database methods according to the present invention can be used to predict gene function and to assist in target validation. Databases that include genetic diversity, i.e., having cellular descriptors from cells of differing genetic backgrounds (tumor, tissue specific, and gene knock out cell lines), can provide the capability to compare cells of unknown genetic background to those in the database. Similarly, the descriptor of an unknown cellular portion in the presence of multiple drugs can be queried against the descriptors of the known markers in the database. For example, if an unknown gene is tagged with Green Fluorescent Protein (GFP), the database may be used to identify the cellular portions for which that unknown gene encodes.

According to the present invention, target validation and specialized cell-based assay screening can be performed using database systems and methods to serve as a universal high-throughput cell-based assay that can evaluate the molecular mechanism of drug action. As new genes are isolated and identified, a large collection of available gene-based knowledge is becoming available. From this large collection of new genes, potential protein targets can be identified using the genomic tools of sequence analysis and expression profiling. However, unless a gene mutation is tightly linked to a disease state, further validation of individual targets is a time consuming process, becoming a bottleneck in drug discovery. Furthermore, robotics and miniaturization are making "High Throughput Screening (HTS)" the industry standard, substantially reducing the time and cost of running a target-based biochemical assay. Therefore, it is now possible to routinely screen large libraries and use a resulting "hit" to validate the target. In such approaches, a specialized cell-based assay would be developed to test hits for each target. Since this often involves

the creation of cell lines expressing new markers, this stage may also become a bottleneck that cannot keep pace with HTS. In addition, these cell-based assays may not be amenable to high-throughput screening, making it difficult to test the increasing number of analogs arising from combinatorial chemistry.

5 In a particular embodiment according to the invention, a rapid characterization of large compound libraries for potential use as pharmaceutical products can be provided by predicting properties of compounds that relate to the compounds' potential as bioactive drugs. In many drug discovery situations, virtually millions of compounds can be passed through a HTS assay against a small number of
10 validated targets. These assays produce hundreds to thousands of potential hits. These hits can then be subsequently screened by a pipeline of secondary and tertiary screens to further characterize their specificity, often time completely missing non-specific interactions with other proteins. Techniques according to the present invention can provide a replacement to such screening operations by providing
15 information about cellular accessibility and mechanism of action for the hits coming from a HTS system. Furthermore, it can replace the biochemical HTS assay and allow rapid and accurate identification of attractive compounds from large libraries without an intervening biochemical assay. The cell information can be predictive of whether to continue into an animal model for each compound, and which animal model to
20 pursue.

The principles of the present specifically contemplate a wide variety of research methodologies, or usage scenarios, implementing these principles. The following discussion of three such scenarios is by way of illustration and not limitation. Study of the principles enumerated herein will render evident to those
25 skilled in the art certain additional methodologies or usage scenarios enabled by the teachings hereof. The present invention specifically contemplates all such modifications. The following description presents some specific embodiments and scenarios that represent a broader use of cellular phenotypic data and characterizations to deduce mechanisms of action and other features of cellular
30 responses to various stimuli. Such procedures generally involve producing a quantitative cellular phenotype based upon two or more cellular attributes and then comparing that phenotype to phenotypes previously stored and indexed. Such

procedures make use of databases or other repositories of biological information. The invention is not limited to the specific embodiments described here.

Considering first the procedure 2000 depicted in Figure 20, a compound has been identified as having a particular cellular activity. See 2004. For example, a compound may be found to inhibit the growth of certain cancer cell *in vitro* by a specific and desired mechanism of action. This may be a particular company's "gold standard."

Next, the compound is analyzed at 2006 in terms of its effect on one or more cell lines. More specifically, the compound is linked, virtually, to a particular phenotype. Two or more values or measures of cellular attributes characterize that phenotype. These attributes are quantified in the context of specific cellular markers.

In one example, the cellular marker is an organelle such as a nucleus or Golgi apparatus. Measured attributes useful for characterizing an associated phenotype include geometric parameters (e.g., size, shape, and/or location of the organelle) and composition (e.g., concentration of particular biomolecules within the organelle).

The phenotype may be characterized by administering the compound of interest to various cell lines and in various concentrations. In each example within this matrix, the attributes of interest are measured. Ultimately, certain phenotypic features (combinations of attribute values) are associated with the compound of interest. These features provide a template for the phenotype.

Next, using the phenotype as identified at 2006, the process identifies other compounds providing similar features. The goal here is to present a list of compounds having a mechanism of action similar to that of the compound that started the process. This allows researchers to identify a mechanism of action, if not already known, for their compound and to draw conclusions based upon their compound's link to other known compounds (which may not be chemically/structurally similar to the compound of interest).

Identifying similar compounds based upon phenotype can take many paths. Most will involve some mathematical basis. For example, the phenotype defined at 2006 can be represented as a fingerprint or vector comprised of multiple scalar values of cellular attributes (as described above). The phenotype representation can then be compared against known phenotypes characterized by the same format

(e.g., they are all characterized as vectors having the same attribute set, but with different values of the attributes). The comparison may be as simple as a Euclidean distance or more sophisticated as a neural network or multivariate statistical correlation.

5 The known compounds and associated phenotypes may be stored as database records or other data structures that can be queried or otherwise accessed as part of the identification procedure. The compounds may also be associated with other relevant data such as clinical toxicity, cellular toxicity, hypersensitivity, mechanism of action, etc. (when available).

10 Compounds found to be sufficiently similar to the starting compound are returned for consideration by researchers. A data processing system may rank such compounds based on degree of similarity to the starting compound. In some cases, the system may even provide similarity scores associated with the listed compounds.

15 Often researchers wish to determine whether their particular compound has clinical or biochemical effects beyond those that they are already aware of. In a typical scenario, the compound of interest was selected based upon its strong binding a target or its stimulation or inhibition of cell growth in a particular cell line. The process associated with 2010 has likely identified the compound of interest as having
20 a particular mechanism of action based on phenotypic similarity to other compounds having a similar mechanism of action. However, within the region of biochemical space, there may be subspaces (characterized by subphenotypes) that correspond to separate properties. For example, within the phenotypic space associated with one mechanism of action, there may be subspaces associated with clinical toxicity,
25 cellular toxicity (likely overlapping the clinical toxicity space), and little or no toxicity. Obviously, a researcher would like to know whether her compound is likely to be toxic.

 Thus, the process 2000 may include characterizing the compound of interest in terms of its distance from (i.e., similarity to) specific phenotypes having
30 known characteristics. In a typical example, the known characteristic is toxicity. This feature allows the researcher to quantify her compound in terms of mechanism of action AND toxicity (or in terms of two or more other relevant properties associated

with phenotype). To allow simple ranking or characterization, compounds of interest may be scored according to a simple or weighted Boolean expression.

A second scenario of interest is depicted in Figure 21. This scenario again defines a phenotype in terms of a quantifiable vector or other measure.

5 However, rather than using a compound of interest to generate the phenotype, some other cellular stimulus is used to generate the phenotype.

As shown, a process 2100 begins with receipt of cells of interest. See 2104. In many situations, the cells are produced by a genetic or epigenetic process that affects the expression level or activity of a particular protein. More generally,
10 any cellular stimulus (e.g., radiation level and type, gravity level, magnetic field, acoustic perturbations, etc.) can be used to generate the cell line of interest. Importantly, this stimulus affects the phenotype and can be correlated therewith.

In the context of drug discovery, a gene encoding for a particular target can be genetically knocked out, underexpressed, overexpressed, expressed in a non-
15 native state, etc. This may be accomplished via standard procedures involving genomic modification, translation or transcription apparatus modification (e.g., use of antisense nucleic acids), blocking target activity (using antibodies to a receptor site for example), and the like. These processes will generally affect the phenotype in some quantifiable way. Importantly, they clearly and unambiguously define a cellular
20 phenotype associated with altering the activity of the target protein.

At 2106, the process involves measuring one or more cellular features from the cell line of interest to define/quantify the phenotype. This may be accomplished as described above with reference to 2006. Next, at 2108, the cellular phenotype generated in this manner is used to identify and rank a set of compounds
25 associated with the phenotype. This operation may proceed in the manner of operations 2008 and/or 2010 from Figure 20.

Finally, at 2110, the process clusters the compounds returned at 2108 by a mechanism of action. The operation 2106 has tightly bound a mechanism of action to a phenotype. Various compounds characterized and stored in a system
30 database may be tentatively assigned a mechanism of action or may have no suggested mechanism of action. By matching their virtual phenotype to the phenotype generated at 2106, one can create or strengthen an association between the compounds and mechanism of action relevant to the stimulus at 2104.

Considering now Figure 22, a third scenario is depicted. This scenario again involves using a virtual phenotype to glean information relevant to a mechanism of action or other cellular activity. In this case, assay data from a group of compounds (e.g., a primary or focused library) is used to elucidate a phenotype.

5 As shown, a process 2200 begins by identifying a target protein. See 2204. Then, at 2206, the process involves identifying positive and negative biochemical hits. More generally, this may involve ranking a number of compounds based upon their interaction with the target. In a specific case, the compounds are ranked based upon their binding affinities to or ability to inhibit the enzymatic activity
10 of the target protein.

After the compounds have been characterized in some manner based upon their interaction with the target, they are used to define a cellular phenotype. See 2208. Generally, the techniques to accomplish are the same as described with reference to operation 2006 of Figure 20. In this case however, one may obtain a
15 strong correlation between mechanism of action (involving the target) and phenotype by using multiple of the compounds identified at 2206. For example, some of the "best hits" may be administered to cell lines in various concentrations. And some of the least effective compounds may also be administered. Cellular attributes that are more strongly exhibited with increasing concentration of the best hits (and not
20 exhibited or exhibited only weakly upon administration of the negative hits) can be used to define the virtual phenotype. In a related approach, compounds having widely varying levels interaction with the target are administered to cells. Those cellular attributes that vary linearly or at least monotonically with the degree of interaction between the target and compound represent attributes that can be used to define the
25 virtual phenotype.

After the cellular phenotype has been defined, previously characterized compounds may be clustered with that phenotype. See 2210. As with operation 2110 of Figure 2, this may create or strengthen an association between a mechanism of action and various compounds in a database.

30 Finally, and optionally, procedure 2200 may provide a "higher resolution" mechanism of action for the compounds identified at 2206. See 2212. Presumably interaction with the target suggests a specific mechanism of action or at least some aspect of a mechanism of action. However, a given target may participate

in a larger cellular mechanism of action – unknown to researchers. Further, a compound may that binds with the target may participate in multiple mechanisms of action – some of which do not involve the target. By linking the target (and its positive hits) to a particular phenotype, some of these additional cellular level activities can be elucidated. The defined phenotype may have been previously identified as associated with other mechanisms of action or higher resolution mechanisms of action. Thus, the phenotype identified at 2208 can be leveraged to generate a higher resolution mechanism of action at 2212.

As suggested in the above discussion, compounds and associated phenotypes may be stored as database records. Such databases can take on many flavors. In one example, a database includes various pieces of information relevant to oncology. Such database may include numerous compounds classified by cellular phenotype, mechanism of action, toxicity, etc. More specifically, the database may include data on commercially available compounds clustered by cellular phenotypes corresponding to mechanisms of action. Further the databases of interest may extended or combined (via standard relational tables and algebra for example) to include additional data such as pharmacology data, cellular genomics data, gene expression data, protein expression data, etc. In a specific example, the database includes measurements made on a subset of the NCI60 cell lines, using DNA, Golgi apparatus, and/or microtubules as markers for defining the phenotypes. Other data includes dosage response information, variation in effect over time, etc. The compounds populating the database could include known National Cancer Institute oncology study compounds. In a specific embodiment, the compound set includes some or all of the compounds mentioned in the article “A gene expression database for the molecular pharmacology of cancer,” Nature Genetics, 24, pp. 236-244 (March 2000).

Various biological analyses may be conducted to develop additional information for characterizing compound mechanisms of action, etc. For example, a cell count analysis may be used to develop dose response curves, GI 50 data, etc. The cell cycle may also be analyzed to find out how various stages in the cycle vary in response to particular stimuli. The Golgi apparatus may be analyzed to determine whether it is in a normal state, a dispersed state, a diffused state, etc. As another example, tubulin may be analyzed to determine whether it is normal, de-polymerized,

over-polymerized, bundled, etc. Obviously, combinations of such analyses may be performed. For example, properties of the Golgi apparatus or tubulin may be analyzed over one or more cell cycles.

In some embodiments, techniques according to the present invention
5 can provide tools for the later stages of drug development such as clinical trial design and patient management. The properties of known drugs, such as clinical trial and patient response information, will be used in a similar fashion as the pre-clinical information to provide predictions about the properties of novel compounds. Because the human cell is the locus of drug action, a database containing drug-cell interactions
10 will be able to provide predictive value for this aspect of drug development.

Although the above has generally been described in terms of specific hardware, software, and methods, it is understood that many alternatives can exist. In particular, the present invention is not limited to a particular kind of data about a cell, but can be applied to virtually any cellular data where an understanding about the
15 workings of the cell is desired. Thus, in some embodiments, the techniques of the present invention could provide information about many different types or groups of cells, substances, and genetic processes of all kinds. Of course, one of ordinary skill in the art would recognize other variations, modifications, and alternatives. Some examples according to the present invention are provided below.

20

EXPERIMENTS

To prove the principle and demonstrate the objects of the present invention, experiments have been performed to determine the effects of manipulations on cell structure using imaging and analysis techniques applied to a variety of
25 situations. These experiments were performed by growing multiple cell lines in the presence of multiple compounds, or substances. Cells were fixed and stained with fluorescent antibodies or labels to multiple cellular portions. One or more images of the cells were then obtained using a digital camera. Descriptors were built by quantifying and/or qualifying patterns of one or more feature from each image in the
30 cell lines under study. A database was built from the descriptors. As the database grows, it should be able to predict the mechanism of action of an unknown drug by comparing its effect with the effects of known compounds or to identify data clusters within large libraries of compounds.

In a first experiment, an automated method to count the number of cells and differentiate normal, mitotic, and apoptotic cells was created.

Approximately, 5,000 HeLa cells were plated per well in a 96 well plate and grown for 3.5 days. The cells were fixed with -20° MEOH for 5 minutes, washed with TBS for 15 minutes, and then incubated in 5 mg/ml Hoechst 33342 in TBS for 15 minutes. Then, 72 images were collected with a 40x objective and 75 ms exposure time.

The analysis was performed on objects that met a certain size criteria that was based on 1) measuring the size of objects in the image that were clearly not cells and 2) excluding the first peak of the area histogram (Fig. 8B values 1-4654).

Histograms of the individual object data were generated for each type of feature. Fig. 8A shows the histogram for average intensity, and Fig. 8B shows histogram data for the area of each object. Fig. 8C shows the scatter plot of the average intensity vs. the area of all of the objects. The pattern of the scatter plot showed an interesting pattern: a large cluster of cells in one region of the graph, with a scattering of object points in other regions. Because mitotic structures are identified as particularly bright objects, most likely due to the biological fact that the chromatin is condensed, the original Hoechst images could be used to identify which cells were either undergoing mitosis, or otherwise looked abnormal. Manual inspection of 917 cells resulted in the classification of each object. Fig. 8D shows a graph where each type of cellular classification is delimited. This graph clearly shows that the mitotic nuclei are brighter than the interphase nuclei. Further, the different phases of the cell cycle can be separated using these two features. Figs. 8E-8F show bar graphs of the average and standard deviations of the areas and average intensities for each cell classification type. These graphs show that interphase nuclei are statistically less bright than mitotic nuclei and that telophase nuclei are statistically smaller than other mitotic nuclei.

Each image was thresholded to an intensity level of 20. A standard area value was set at 9500 pixels. Automated information gathering about all of the objects was done and collected into an Excel spreadsheet (for more information see, section on imaging system). The following information was recorded:

IMAGE NAME
OBJECT #

AREA
STANDARD AREA COUNT
PERIMETER
FIBER LENGTH
FIBER BREADTH
SHAPE FACTOR
ELL. FORM FACTOR
INNER RADIUS
OUTER RADIUS
MEAN RADIUS
AVERAGE INTENSITY
TOTAL INTENSITY
OPTICAL DENSITY
RADIAL DISPERSION
TEXTURE DIFFERENCE MOMENT
EFA HARMONIC 2, SEMI-MAJOR AXIS
EFA HARMONIC 2, SEMI-MINOR AXIS
EFA HARMONIC 2, SEMI-MAJOR AXIS
ANGLE
EFA HARMONIC 2, ELLIPSE AREA
EFA HARMONIC 2, AXIAL RATIO
EFA HARMONIC 3, SEMI-MINOR AXIS

The following results were obtained:

- 1,250 objects were counted
- 201 of those objects has standard area counts > 2 (area > 19000 pixels)
- 195 objects had areas < 6000 pixels
- 1529 objects estimated in total
- 1328 object areas are > 6000 pixels
- The data was reduced to 917 objects that were $6000 < \text{area} < 19000$
- For the 917 objects a scatter plot of area vs. average intensity and a histogram of the average intensity were generated.

- 116 objects that had average intensity intensities > 60 were manually looked at to determine their morphology.

- Of those 116 objects:

6 were dead or indistinguishable

4 were interphase

30 were prophase

32 were metaphase

24 were anaphase

20 were telophase (10 pairs)

10

- 12 prophase objects were missed because of gray scale cut off. (8 of those prophase cells had gray scale values > 57 , as did 7 interphase)
- 1 telophase object was missed because it was too small (< 6000)
- 1 prophase object was missed because it was too big (> 1900)
- 16 mitotic objects were missed because they were parts of objects with standard count > 2 .

15

In sum, out of 917 single objects, the analysis correctly identified 106 out of 130 mitotic objects, or (81% predictive, 91% of identified mitotics). Out of 917 single objects, the analysis incorrectly identified only 10 non-mitotics as mitotics (1% total, 8% of identified mitotics); 14 mitotics as interphase (1.4% total, 1% interphase). An automated classification system that would automatically assign values to each object using these or other measurement features can thus be developed, utilizing the principles set forth herein.

In a second experiment, the effects of Taxol on MDCK cells and the different types of morphological effects were observed. A plurality of MDCK cells grown in 96 well plates were treated with Taxol for 4.5 hours at different concentrations (10 uM-1pM). They were then fixed, labeled with Hoechst, and imaged.

This experiment used a labeling protocol comprising: MEOH fix at – 20°, Wash in PBS, Block in PBS/BSA/Serum/Triton-X 100, Incubate with 5 µg/ml Hoechst 10 minutes, and wash.

Cells were inspected for different morphologies and manually counted at each different drug concentration in one well. Fig. 9 shows example images from each drug concentration and the different types of morphologies and cells are highlighted. Fig. 10 shows the distribution of each morphology within the cell population as a function of drug concentration. The higher the concentration of Taxol, the larger proportion of cells underwent apoptosis, and the fewer number of normal mitotic cells were detected.

In a third experiment, the purpose was to determine whether the automated analysis methods developed in the first experiment can detect differences in Hoechst morphology in the presence of 6 known compounds at one concentration and exposure time in one cell line. In this experiment, HeLa cells were separately treated with 6 compounds with known mechanism of action. The quantitative methods described in the first experiment were applied to the Hoechst images.

Approximately 5,000 HeLa cells per well were plated in a Costar black-walled 96 well tissue culture treated plate and left to recover in the incubator for 24 hours. After this time, 10 ug/mL of cytochalasin D (CD), Taxol, hydroxyurea, vinblastine, nocodazole, and staurosporine was added to different wells at a 1:100 addition in DMSO.

The cells were incubated in the presence of drug for 24 more hours. After 24 hours, the cells were removed and fixed as in the first experiment. Then, 9 images per well were collected of the Hoechst staining using a 10x objective.

5 The low magnification images taken of Hoechst were run through the automated image analysis method described in the first experiment. Plots of the average intensity and area were made of each compound. Fig. 11 shows the scatter plots of the compounds. The scatter plots of each compound are visually distinct. For example, cells treated with CD are smaller than control, and cells treated with Hydroxyurea are larger and brighter. Furthermore, the number of cells per well was
10 very different (data not shown).

The effects of different compounds can be clearly and automatically distinguished by identifying changes in cellular morphology. This method can also be used to count adherent cells.

The next experiment was to develop clustering algorithms that assign
15 statistically meaningful values to the representative two dimensional data shown in Fig. 10, and even more complicated clustering of all of the multidimensional data that can be extracted across one, and multiple images.

A fourth experiment was performed to obtain high magnification images of two markers in the presence of drugs. In this experiment, HeLa cells were
20 treated with 80 generic compounds with known mechanism of action. The quantitative methods described in the first experiment were applied to the Hoechst images.

Approximately 5,000 HeLa cells per well were plated in a Costar black walled 96 well tissue culture-treated plate and left to recover in the incubator for 24
25 hours. After this time, 10 ug/mL of each compound from the Killer Plate from Microsource Discovery Systems (Gaylordsville, CT) was added to different wells at a 1:100 addition in DMSO. The cells were incubated in the presence of drug for 24 more hours. After 24 hours, the cells were removed and fixed as in the first experiment. In addition to being labeled with Hoechst 33342 (against chromatin),
30 cells were also labeled with 1 unit of rhodamine-conjugated phalloidin (against actin) for 30 minutes.

The 96 well plate was imaged twice. Once, 9 images per well were collected of the Hoechst staining using a 10x objective. After this, one image per well of both the phalloidin and Hoechst staining was collected using a 40x objective.

The resulting high magnification images were analyzed qualitatively and distinct pattern differences were detected in both the Hoechst and phalloidin images. Fig. 12 shows three example images from the experiment. The top row is the Hoechst staining, and the bottom row is the phalloidin staining from the same well. The columns show the images from wells treated with just DMSO (control), cytochalasin D, and Colchicine. The morphology of each marker is different in the presence of each drug. Interestingly, there is an effect in the morphology of the chromatin in the Hoechst image of cytochalasin D, which directly targets the actin cytoskeleton (and thus there is an expected effect in the phalloidin image). Also, there is an effect on the actin cytoskeleton, compared to control, in the presence of colchicine that directly targets the microtubule network.

The low magnification images were analyzed as described in the first experiment, and different patterns were seen in both the average intensity vs. area plots, and in the number of cells per well (data not shown). Thus, changes in patterns of a marker that is "down-stream" from the direct target of a compound are detectable. Automated image analysis protocols for actin and other markers can be developed similarly, again utilizing the principles set forth herein.

A fifth experiment was performed to test quadruple labeling of 9 different cell lines grown in normal conditions. In this experiment, NCI-H460, A549, MDA-MD-231, MCF-7, SK-OV-3, OVCAR-3, A498, U-2 OS, and HeLa cells were plated. Then, the cells were fixed and stained for portions of the each cell known as DNA, tubulin, actin, and Golgi.

The following table summarizes the procedures for this experiment:

Action	Active Ingredient/Notes	Buffer	Vol/ well	Desired Time	Temp
Remove media	NOTE: gently by pipetting, not aspiration				
Fix	4% Formaldehyde	PBS	100µl	20 min	rt
Wash		TBS	100µl	5 min	rt
Wash		TBS	100µl	5 min	rt

Permeabliz e	0.1% Triton X-100	TBS	100μl	10 min	rt
Permeabliz e	0.1% Triton X-100	TBS	100μl	10 min	rt
Block	% BSA % Serum Filter sterilize before use	TBS w/azide	100μl	1hr or o/n	rt or 4°C
Primary Antibody	1:1000 dilution of DM1α	TBS + 1% BSA + 0.1% TX-100	50μl	1hr or o/n	rt or 4°C
Wash		TBS	100μl	5 min	rt
Wash		TBS	100μl	5 min	rt
Wash		TBS	100μl	5 min	rt
Fluorescent Stain	FITC lens culinaris 1:500 Rhodamine-Phalloidin 1:500 CY5 goat anti-mouse 1:100	TBS + 1% BSA + 0.1% TX-100	50μl	1 hr.	rt, dark
Wash		PBS	100μl	5 min	rt, dark
Hoechst	1:1000 dilution of 5mg/ml	TBS	100μl	15 min	rt, dark
Wash		PBS	100μl	5 min	rt, dark
Wash		PBS	100μl	5 min	rt, dark
Wash		PBS	100μl	5 min	rt, dark
Store		PBS	200μl	1 month	4°C

Cells were plated out at different densities for 48 hours. Cells were fixed and labeled by the above method. Cells were imaged using an automated imaging system that collected 9 images from each marker using a 10x objective.

Higher magnification images were collected of a few cells for demonstration purposes.

In this experiment, each cell line demonstrated different morphological patterns as determined by phase. For example, A549 cells are much more compacted than OVCAR-3 cells as determined by phase contract imaging (data not shown). The different fluorescent markers showed even bigger differences between different cell lines. Figs. 13 and 14 show 4 panels of each marker for A549 (Fig. 13) and OVCAR-3 cells (Fig. 14). The markers are Hoechst (upper left), Phalloidin (upper right), Lens culinaris (lower left), and DM1a antibody (lower right). The following table summarizes the qualitative differences between these images:

MARKER	A549	OVCAR3
Hoechst/DNA	small	large
Phalloidin/actin	fuzzy	crisp - many stress fibers
Lens culinaris/Golgi	compact	Disperse/punctate
DM1alpha/Tubulin	perinuclear	evenly distributed

Higher magnification images were taken of the OVCAR3 cells. Fig. 15 shows the same markers at 20x, and Fig. 16 shows the markers at 40x. While the highest magnification images show the most detail, these images illustrate that very little morphological or feature information is lost in the 10x images.

These data exemplify the differences in morphology seen between different cell types. Thus the automated image analysis software can be customized for each marker in each cell type. Different drugs should effect these morphologies differentially.

An automated quantification method for each marker and cell line can be similarly developed.

A sixth experiment was conducted with a more sophisticated software package and to develop more flexible image recognition algorithms. In this experiment, prototype image features extraction was performed using MatLab programming language with image toolbox and SDC morphology toolboxes. Algorithms are being developed that will automatically identify objects on images and

to measure various morphological and feature parameters of these objects. Many different features for each of the cellular markers were acquired.

An example of a MatLab program called "AnalyseDNA" that takes as an input an unlimited number of images, identifies individual objects in these images
 5 based on either their intensities, or based on edge-detection algorithms, and extracts a number of morphological and intensity characteristics of these objects. A copy of this program follows:

Listing of the AnalyseDNA.m program and of some of the supporting subroutines

```

10 function files_analysed = AnalyseDNA(filemask, outpath,
    nx, ny, filter_range, dext, modifier, sfname)
    % AnalyseDNA performs measurements on files of DNA images
    % V1. EV 2-11-99; 2-15-99; 2-16-99
15 %
    % files_analysed = AnalyseDNA(filemask, outpath, nx, ny,
    filter_range, dext, modifier, sfname)
    %
    % PARAMETERS:
20 %     ALL PARAMETERS ARE OPTIONAL
    %
    %     FILEMASK - mask for file names to be analyzed
    INCLUDING PATH(for example c:\images\*.tif)
    %     DEFAULT '.*.tif' (all *.tif files in the current
25 directory).
    %
    %     OUTPATH - path to a directory where all the output
    files will be placed.
    %     DEFAULT - output is saved in the same directory
30 which contains images
    %
    %     NX, NY - number of individual images in montage
    images along X and Y axes (DEFAULT 1)
    %
  
```

```
%    FILTER_RANGE - 3 col-wide array (or[]). Specifies
how data is filtered when summary is calculated
%    this parameter internally is passed to GetDNADData
and then to GetSummaryData - see these
5 %    functions for details. For example: [2 2 Inf; 6 100
8000] will case all raws of data for which
%    values in column 2 are less than 2 and all raws
where values in column 6 are less than 100 or
%    more than 8000 to be excluded from all
10 calculations of a summary.
%    DEFAULT - [] (means do not filter, summarize all
data)
%
%    DEXT - string. Extension for data files being saved.
15 %    DEFAULT 'dat';
%
%    MODIFIER - this modifier is 'SUMMARY', summary file
is created;
%    'SUMMARY ONLY' - only summary is generated,
20 data for individual files are not saved
%
%    sfname - string. File name of a summary file
%    DEFAULT 'summary[date].dat'
%
25 % OUTPUT:
%
%    AnalysedDNA works on image files or montages. For
each image file it creates a tab-delimits file of
measured
30 %    parameters of all the objects in the montage with
the same base name as a montage file and extension
specified
```



```
%    by dext parameter (or .dat by default) and file
'errors[date].err' - with the list of files that matched
the
%    filemask but could not be processed.
5 %    If 'summary' or 'summary only' modifier is
specified, it also creates a single file
'summary[date].dat' (or
%    different extension, if specified by DEXT) which
contains summary information for all analyzed files.
10 %
%    ALL OUTPUT FILES are saved in a directory specified
by OUTPATH parameter
%
%    RETURNS *files_analysed* - number of files that have
15 been successfully processed.
%
%    Column designations in the output files are
described in GetDNADData
%
20 % FILE NAME CONVENTIONS
%    AnalyseDNA attempts to identify a number for each
file to identify the file in summary output.
%    It does that by looking for the first space or
underscore, followed by a number and then takes
25 %    as many successive numbers as it can find. If it
fails to identify a number it assigns a
%    default which is -1
%
%
30 % SEE ALSO GetDNADData, GetSummaryData
%
% TO DO    improve error handling in opening and writing
files (GLOBAL error_file ?)
```

```
%          include procedures for writing text headers
into the output files

if nargin > 8
5   error ('Wrong number of input parameters');
end
if nargsout >1
    error ('Wrong number of output parameters: only one
allowed');
10 end

% set defaults
need_summary = 0;
summary_only = 0;
15 use_default_outpath = 0;
datestring = datestr(floor(now));
if nargin == 7      % set default summary file name
    sfname = ['summary' deblank(datestring)]; % extension
will be appended later based on dext
20   if deblank(upper(modifier)) == 'SUMMARY'
        need_summary = 1;
    elseif deblank(upper(modifier)) == 'SUMMARY ONLY'
        need_summary = 1;
        summary_only = 1;
25   else
        error (['Wrong parameter: unknown modifier '
modifier]);
    end
end
30
if nargin == 5
    % default data file extension
    set dext = 'dat';
end
```

```
    if nargin == 4
        % default filter range
        filter_range = [];
    end
5   if nargin == 3
        ny = 1; % default number of images in montage along Y
    end
    if nargin == 2
        nx = 1;
10   end
    if nargin == 1
        use_default_outpath = 1;
    end
    if nargin == 0
15     filemask = '*.tif'
    end

    % check parameters
    if ( ~ischar(filemask) | ~ischar(dext) | ~ischar(sfname)
20   )
        error('Wrong parameter type: filename, filepath,
dext and sfname should be strings');
    end
    if ( ( size(nx) ~= [1 1] ) | ( size(ny) ~= [1 1] ) )
25     error ('Wrong parameter type: nx and ny should be
scalars (1x1 arrays)');
    end
    if (~isempty(filter_range) & size(filter_range, 2) ~= 3)
        error ('Wrong parameter type: filter range should be
30  [] or 3 - cols-wide array');
    end
    % end testing parameters

    % Generate list of files to process
```

```
datapath = getpath(filemask);
if use_default_outpath == 1
    outpath = datapath;
5  end
if exist(outpath, 'dir') ~= 7
    error(['Path ' outpath, 'not found. Exiting..']);
elseif exist(datapath, 'dir') ~= 7
    error(['Path ' datapath, 'not found. Exiting..']);
10 end

sfname = makefullname(outpath, sfname, dext);
if need_summary == 1
    if exist(sfname, 'file')
15     disp(['File ', sfname, 'already exists!']);
    input ('Press ^C to abort, Enter to delete and
continue');
    delete(sfname);
    end
20 end

flist = FileList(getfname(filemask), datapath);
numfiles = size(flist, 1); % total number of files to
25 process
disp(['About to process ', num2str(numfiles), ' files']);
%DEBUG - commented out "input" to run from Wrod
input('Press ^C to abort, Enter to continue');

30 % main loop where the job gets done:
error_file = makefullname(outpath, ['error' datestring
'.err']);
num_processed = 0;
num_error = 0;
```

```

for i = 1:numfiles
    % first generate file name for a data output file
    current_fullname = flist(i, :); % full name with path
    and extension
5    current_datafile = makefullname(outpath,
    makefname(getbasefname(current_fullname), dext) );

    %extract number from a filename
    fnumber = getfilenumber(current_fullname);
10

    % load an imagefile, record errors
    read_error = 0;
    try
        I = imread(current_fullname);
15        %DEBUG
        disp(['Image file #', num2str(fnumber), '
loaded']);
        catch
            % record file-opening error in an error_file
20            read_error = 1;
            num_error = num_error +1;
            msg = [current_fullname ': ' lasterr];
            add_error_msg(error_file, msg);
        end
25

    % extract and write data to a file in outpath
    if read_error ~=1
        if (need_summary == 0)
            %DEBUG
30            disp(['Starting analysis of file #',
            num2str(fnumber), '.']);
            current_data = GetDNADData(I, nx, ny, fnumber);
            %DEBUG

```

```

        disp (['Finished analysis of file #',
num2str(fnumber), '.']);
        %load current_data.mat 'current_data';
        write_data(current_data, current_datafile);
5      else      %summary needed
            %DEBUG
            [current_data, current_summary] = GetDNADData(I,
nx, ny, fnumber, filter_range);
            %load current_data.mat 'current_data';
10          %load current_summary.mat 'current_summary';
            write_summary (current_summary, sfname);
            if summary_only ~= 1
                write_data(current_data, current_datafile);
            end
15      end
    end
end % of the main for loop
num_processed = numfiles - num_error;

20 %=====end function AnalyseDNA()
=====

%=====
=====

25 function result = add_error_msg(filename, msg)
    % adds string MSG to an errorfile FILENAME
    % returns 1 if success, 0 if failure

    err_FID = fopen(filename, 'at');
30 if err_FID == -1
        warning(['Can not open error file ' filename]);
    else
        fprintf(err_FID, '%s\n', msg);
        fclose(err_FID);

```

```

end
%=====end function add_error_masg()
=====

5 %=====
=====

function N = getfilenumber(fname)
% returns the first number extracted from a file name
(string) or -1 if fails to extract any number
10 numbers = NumbersFromString( getfname(fname) ); % vector
of all numbers encoded in the name

                                % (but not in the path, even if
present)
15 if isempty(numbers)
    N = (-1);    % return -1 if no numbers found in the
name
else
    N = numbers(1);
20 end

%===== end function getfilenumber()
=====

25 %=====
=====

function result = write_data(data_array, file_name)
% writes data in a data_array in a tab-delimited ascii
file.
30 % result is 0 if success and -1 if failure
% if file_name exists, overwrites it
result = -1;
try
    fid = fopen(file_name, 'wt');

```

```

        if fid ~= -1
            for k = 1:size(data_array, 1)
                fprintf(fid, '%g\t', data_array(k, :));
                fprintf (fid, '\n');
5         end
            test = fclose(fid);
            result = -1;
        catch
            result = -1;
10    end

%===== end function write_data()
=====

15 %=====
=====
function result = write_summary (s_vector, file_name)
% appends summary vector s_vector to a file_name (ASCII
tab-delimited file).
20 % if file_name does not exist, creates it.
% result is 0 if success and -1 if failure
%
result = -1;
try
25     % debug
        fid = fopen(file_name, 'at');
        result = fprintf(fid, '%g\t', s_vector);
        result = fprintf(fid, '\n');
        result = fclose(fid);
30     result = 0;
    catch
        result = -1;
    end
end

```



```

% ===== end function write_summary()
=====

function Data = GetObjectsData(I, Ilabel)
5 % GetObjectsData returns array measurements of objects in
  image "I" masked by "Ilabel"
  % EV 2-3-99; 2-10-99
  % OData = GetObjectsData(I, Ilabel) returns an array of
  morphological and intensity measurements
10 %   taken from a grayscale image "I". Objects are
  identified on a mask image Ilabel, usually
  %   created by bwlable()
  % OUTPUT:
  % Each row in the output array OData represents
15 individual object
  % columns contain the following measurements:
  %
  %   1 - Index ("number" of an object);      8 -
  Solidity;
20 %   2 - X coordinate of the center of mass; 9 - Extent;
  %   3 - Y coordinate      "-"; 10 - Total
  Intensity;
  %   4 - Total Area (in pixels);              11 - Avg.
  Intensity;
25 %   5 - Ratio of MajorAxis/MinorAxis;        12 - Median
  Intensity;
  %   6 - Eccentricity;                        13 - Intensity of
  20% bright pixel
  %   7 - EquivDiameter;                      14 - Intensity of
30 80% bright pixel
  %
  % For details on morphological parameters see information
  on MatLab imfeature();

```

```
% Intensity parameters are either obvious or are
documented in comments in this file.

% Procedures in this file are documented in notebook file
"MATLAB Measuring Nuclei (1) 1-29-98.doc"

5
if (nargin ~= 2)
    error ('function requires exactly 2 parameters');
end
if (nargout ~= 1)
10    error ('function has 1 output argument (array X by
    14)');
end

% finished checking arguments

15
% first collect morphological parameters in a structure
array:
ImStats = imfeature(Ilabel, 'Area', 'Centroid',
    'MajorAxisLength',...
20    'MinorAxisLength', 'Eccentricity', 'EquivDiameter',
    ...
    'Solidity', 'Extent', 8 );

% now convert it into array (matrix) while collecting
25 intensity data for each object:

%preallocate output array:
numobjects = size(ImStats, 1);
OData = zeros(numobjects, 14);

30 %now convert ImStats into array and add intensity data to
it
for k=1:numobjects
    OData(k, 1) = k;
    OData(k, 2) = ImStats(k).Centroid(1);
```

```

        OData(k, 3) = ImStats(k).Centroid(2);
        OData(k, 4) = ImStats(k).Area;
        OData(k, 5) = (ImStats(k).MajorAxisLength) /
        (ImStats(k).MinorAxisLength);
5         OData(k, 6) = ImStats(k).Eccentricity ;
        OData(k, 7) = ImStats(k).EquivDiameter;
        OData(k, 8) = ImStats(k).Solidity;
        OData(k, 9) = ImStats(k).Extent;

10         % now collect and assign intensity parameters from
        image I

        object_pixels = find( Ilabel == k);
        object_area = size(object_pixels, 1); %same as total
15 number of pixels in the object
        object_intensities = double(I(object_pixels)); %
        need to convert to double to do math
        sorted_intensities = sort(object_intensities); %
        will need to get median, 20% and 80% pixels
20         total_intensity = sum(object_intensities, 1);
        avg_intensity = total_intensity / object_area;
        median_intensity = sorted_intensities( floor(
        object_area/2 ) + 1 );
        pix20 = sorted_intensities( floor(object_area*0.2)+1
25 ) ; %brightest pixel among dimmest 20%
        pix80 = sorted_intensities( floor(object_area*0.8)+1
        ) ;

        OData(k, 10) = total_intensity;
30         OData(k, 11) = avg_intensity;
        OData(k, 12) = median_intensity;
        OData(k, 13) = pix20; %brightest pixel among dimmest
20%

```

```

        OData(k, 14) = pix80; %dimnest pixel among brightest
    20%
    end %for

5   %===== end function
    GetObjectsData() =====

function Imask = MaskDNA1(I);
10  % MaskDNA1 - generates binary mask for cell nuclei
    through edge detection
    % EV 1-22-99; 2-6-99; 2-10-99
    % Imask = MaskDNA1(I)
    % PARAMETERS
15  %   I - intensity image (grayscale)
    % OUTPUT
    %   Imask - BW image with objects from I
    %
    % For more details see Notebook Matlab_DNA_masking1_1-22-
20  99.doc
    % Uses SDC Morphology Toolbox V0.7

    if (nargin ~= 1)
        error('Wrong number of input parameters');
25  end
    if (nargout ~= 1)
        error('Wrong number of output parameters: one output
        argument should be provided');
    end
30

    Imask = edge(I, 'canny');
    Imask = mmdil(Imask, mmsecross(1));
    Imask = mmero ( mmclohole(Imask,mmsecross(1)));

```

```

Imask = mmedgeoff(Imask, mmsecross(1));
% note that mmedgeoff this command removed FILLED OBJECTS
but not touching OUTLINES.
% these outlines can be removed by filtering:
5  Imask = medfilt2(Imask, [5 5]);

%=====end MaskDNA1 =====

```

Given the list of image files or montages of images as an input, this
 10 program creates an individual file for each image that contains the following
 quantitative measurements for all objects identified in the image:

1 - Index ("number" of an object);	8 - Solidity;
2 - X coordinate of the center of mass;	9 - Extent;
15 3 - Y coordinate "-";	10 - Total Intensity;
4 - Total Area (in pixels);	11 - Avg. Intensity;
5 - Ratio of MajorAxis/MinorAxis;	12 - Median Intensity;
6 - Eccentricity;	13 - Intensity of 20% bright pixel
7 - EquivDiameter;	14 - Intensity of 80% bright pixel

20 A fragment of an output for a single file, containing 9 images of cells
 stained for DNA and acquired with a 10x objective. A montage image that was used
 as a source to generate data in A is presented in Fig. 17.

The same program also summarizes measurements across many files
 and performs statistical analysis of the summary data. It creates a summary file with
 25 the following data:

1 - Image file number;	
2 - Average object Area (in pixels);	3 - STD (standard deviation) of
2;	
30 4 - Avg. of Ratio of MajorAxis/MinorAxis;	5 - STD of 4;
6 - Avg. Eccentricity;	7 - STD of 6;
8 - Avg. EquivDiameter;	9 - STD of 8;
10 - Avg. of Solidity;	11 - STD of 10;

- | | |
|---|----------------|
| 12 - Avg. of Extent; | 13 - STD of 11 |
| 14 - Avg. of objects Total Intensity; | 15 - STD of 14 |
| 16 - Avg. of objects Avg Intensity; | 16 - STD of 15 |
| 18 - Avg. of objects Median intensity; | 19 - STD of 18 |
| 5 20 - Avg. of objects intensity of 20% bright pixel; | 21 - STD of 19 |
| 22 - Avg. of objects intensity of 80% bright pixel; | 23 - STD of 21 |

An example of summary output obtained by running AnalyseDNA against 10 montage files also is shown in Appendix B.

10 A seventh experiment was conducted in order to use sequence analysis algorithms to analyze features of cell images. In this experiment, HeLa cells were treated for 24 hours with several different compounds, and then fixed, and stained with a fluorescent DNA dye. One image of these cells was acquired for each of the treatments and morphometric parameters and features were measured:

15 Resulting measurements were arranged into a string of numbers and reduced to a pseudo- nucleic acid sequence using following rules: At any given position in the sequence a number was substituted by "t" (a code for thymidine) if its value is among highest 25% of the values at the corresponding position in the data set, "g" if it is between 50% and 25%, "c" if it is between 75% and 50%, and "a" if it
20 belongs to lowest 25% of values. Thus one descriptor or sequence was generated per treatment as illustrated in Fig. 18.

Resulting sequences were clustered using an AlignX module commercial software package Vector NTI (<http://informaxinc.com>), which uses a Neighbor Joining algorithm for sequence clustering.

25 The resulting dendrogram is presented in Fig 18. On the dendrogram the closest "leafs" correspond to the closest pseudo-sequences. Interestingly, compounds with similar mechanisms of action cluster together on the dendrogram. Another example of the generation of pseudo-sequences and clustering is shown in Fig. 19.

30 In some embodiments, techniques according to the present invention can provide tools for the later stages of drug development such as clinical trial design and patient management. The properties of known drugs such as clinical trial and patient response information will be used in a similar fashion as the pre-clinical

information to provide predictions about the properties of novel compounds. Because the human cell is the locus of drug action, a database containing drug-cell interactions can be able to provide predictive information for this aspect of drug development.

Although the above has generally described the present invention
5 according to specific systems, the present invention has a much broader range of applicability. In particular, the present invention is not limited to a particular kind of data about a cell, but can be applied to virtually any cellular data where an understanding about the workings of the cell is desired. Thus, in some embodiments, the techniques of the present invention could provide information about many
10 different types or groups of cells, substances, and genetic processes of all kinds. Of course, one of ordinary skill in the art would recognize other variations, modifications, and alternatives.

APPENDIX A

EV Table 1.doc

Example of the output of AnalysedNA.m program
(measurements for a single 3 by 3 montage image)

File	Subimage	object	X coord	Y coord	Area	Area ratio	Eccentricity	Equidiam	Solidity	Extinct	Intensity	Avg. Intensity	Median Intensity	201 pla.	201 pla.
1	1	1	12.2897	152.635	145	1.17293	0.527621	13.5875	0.923367	0.739796	4605	31.7396	31	23	37
1	1	2	16.352	416.032	125	1.60394	0.182471	12.6157	0.903991	0.78135	4606	36.848	38	30	45
1	1	3	20.1073	73.8079	177	1.09485	0.413785	15.0121	0.917098	0.691406	4769	26.9435	29	22	31
1	1	4	21.6186	402.744	47	1.36215	0.478004	7.39978	0.914594	0.739457	3690	85.114	87	67	105
1	1	5	27.0918	164	96	1.30887	0.443134	11.0558	0.888899	0.671329	4502	46.8958	45	38	56
1	1	6	30.3252	359.534	206	2.31106	0.803709	16.1953	0.927928	0.715278	4380	30.9705	32	24	37
1	1	7	33.6629	167.573	89	1.24884	0.471696	10.6451	0.927083	0.714657	4225	47.4719	50	38	44
1	1	8	35.0411	16.9726	146	1.25176	0.401495	13.6347	0.929926	0.746919	5415	37.089	40	28	44
1	1	9	37.746	366.021	47	1.84062	0.439542	7.3578	0.87037	0.632778	6667	141.951	142	113	171
1	1	10	49.1078	170.004	232	1.90491	0.451127	17.187	0.852941	0.703003	5932	42.3793	45	33	51
1	1	11	56.0769	126.534	221	1.95704	0.455955	16.7746	0.924686	0.683335	7040	31.8552	33	25	31
1	1	12	57.7755	41.9932	147	1.33627	0.463301	13.6009	0.907407	0.706771	4745	32.415	34	26	39
1	1	13	52.1444	366.851	171	2.27223	0.897553	14.7555	0.872449	0.706412	5718	54.8421	56	43	68
1	1	14	56.4079	282.232	208	1.92782	0.851944	15.3553	0.923367	0.64375	7137	34.6456	37	28	41
1	1	15	57.0648	277.176	308	1.73485	0.813089	11.7265	0.913254	0.701299	4644	43	43	51	
1	1	16	66.1714	333.161	315	1.13194	0.437266	20.0267	0.75	0.528756	13151	48.0984	50	36	42
1	1	17	65.1409	402.414	220	1.70147	0.80906	16.7366	0.920502	0.617059	9809	44.5864	46	35	54
1	1	18	71.8449	402.13	185	1.74678	0.824583	15.3476	0.91133	0.619123	6124	33.1027	35	25	39
1	1	19	71.626	184.854	123	1.71580	0.817622	12.5143	0.911111	0.723229	4861	39.3577	41	30	47
1	1	20	77.4869	132.513	306	1.6379	0.791981	19.7386	0.822321	0.622231	14559	47.5784	50	38	57
1	1	21	78.7377	208.27	122	1.3157	0.467941	12.4634	0.910448	0.735954	4483	36.7459	40	29	47
1	1	22	81.7866	57.5612	117	1.44713	0.791616	12.2053	0.846364	0.62857	4686	40.0513	43	37	63
1	1	23	84.7392	281.534	373	2.17388	0.867916	21.7926	0.843691	0.513239	16109	43.1877	46	31	52
1	1	24	86.1645	341.976	65	1.20799	0.450991	10.4031	0.846289	0.708333	549	53.9882	57	43	61
1	1	25	86.1408	176.231	143	1.35273	0.717545	13.4935	0.910789	0.794444	4878	34.1119	35	27	41
1	1	26	91.4229	276.924	170	1.36852	0.692619	16.7123	0.833333	0.933333	4933	29.0176	30	23	35
1	1	27	97.7604	317.765	288	1.92553	0.822119	15.1492	0.919721	0.66316	10663	37.0243	39	29	45
1	1	28	85.5841	370.343	113	1.09825	0.452109	11.9948	0.856825	0.668635	4560	40.177	43	33	48
1	1	29	85.992	248.602	118	1.2774	0.822219	12.2573	0.945736	0.746233	4873	41.2866	43	32	51
1	1	30	103.37	155.502	134	1.3208	0.753111	13.0619	0.917808	0.671719	4358	32.5224	34	27	38
1	1	31	103.37	155.502	134	1.3208	0.753111	13.0619	0.917808	0.671719	4358	32.5224	34	27	38
1	1	32	105.346	37.1271	118	1.90329	0.450449	12.2573	0.907692	0.694118	4695	39.7181	42	30	46
1	1	33	121.532	170.645	141	1.57045	0.757011	13.988	0.927632	0.719375	6249	45.5857	49	37	57
1	1	34	125.532	170.645	141	1.57045	0.757011	13.988	0.927632	0.719375	6249	45.5857	49	37	57
1	1	35	128.98	60.3355	152	1.75689	0.822208	11.9116	0.921212	0.767677	6075	45.2303	47	36	54
1	1	36	137.083	129.083	264	1.445	0.840315	16.4033	0.796409	0.536462	9810	36.8797	38	26	45
1	1	37	130.902	111.5	164	1.19276	0.698312	15.4204	0.937143	0.788462	7337	44.7378	47	35	53
1	1	38	132.739	352.345	167	1.19705	0.698312	15.4204	0.935	0.799145	5227	27.9319	27	22	34
1	1	39	135.615	16.6154	13	1.15921	0.50539	4.06843	0.928371	0.8125	94	7.23077	8	3	11
1	1	40	136.571	209.059	101	1.17013	0.450546	11.3601	0.87069	0.674336	7293	72.2079	74	58	88
1	1	41	136.553	37.0909	23	1.21169	0.464506	6.82024	0.916667	0.785116	1949	59.0606	60	44	75
1	1	42	140.831	102.008	121	1.17187	0.736306	12.4122	0.916667	0.733333	1808	39.7355	40	32	47
1	1	43	144.864	89.8199	272	1.15519	0.81825	18.4097	0.931708	0.735333	10814	39.7574	41	32	47
1	1	44	147.093	226.323	161	1.3071	0.61937	16.3175	0.925299	0.715556	9000	55.9006	56	44	68
1	1	45	151.44	256.124	224	1.08008	0.378772	16.448	0.937276	0.777778	9654	43.0992	45	33	53
1	1	46	155.688	171.546	141	1.39135	0.495293	13.9980	0.911569	0.801336	7129	54.8156	57	44	65
1	1	47	160.755	342.354	48	1.71551	0.812532	7.8164	0.857143	0.592593	609	137.488	139	103	168
1	1	48	168.878	11.7677	158	1.42126	0.710595	15.0777	0.933862	0.733333	5931	50.1566	50	38	62
1	1	49	169.513	136.267	217	1.74718	0.820008	16.4221	0.825095	0.5425	7980	36.7742	38	28	43
1	1	50	176.014	356.14	222	1.41888	0.720419	16.8125	0.790036	0.619449	5626	43.3604	47	33	54
1	1	51	177.188	192.393	116	1.13784	0.47709	16.8125	0.900763	0.699225	4671	39.5447	42	32	47
1	1	52	177.181	210.874	127	1.16399	0.487604	12.7162	0.90209	0.755952	4862	38.2435	40	30	45
1	1	53	178.367	410.524	170	1.42122	0.52423	13.4808	0.94528	0.75	9096	41.8776	47	50	77
1	1	54	182.4	392.476	147	1.57768	0.755387	14.7123	0.93927	0.716386	5315	54.9118	58	44	61
1	1	55	189.388	262.719	216	1.31704	0.474923	15.7973	0.916667	0.742424	5020	25.4633	27	20	31
1	1	56	200.744	93.7418	211	1.36993	0.462428	16.4402	0.916667	0.747368	8645	45.7256	48	38	53
1	1	57	199.158	156.725	91	1.04653	0.283161	10.7441	0.90501	0.752086	4188	46.032	47	36	52
1	1	58	205.47	185.871	244	1.36083	0.804182	18.334	0.916667	0.6	9736	37.2977	39	29	46
1	1	59	208.752	70.0435	230	1.63123	0.804718	17.1127	0.946502	0.804196	9764	42.4609	44	34	53
1	1	60	212.584	366.455	193	1.33679	0.525867	13.3374	0.932153	0.727445	7014	25.8918	26	19	29
1	1	61	220.356	30.6227	154	2.47662	0.927388	15.7163	0.94843	0.840054	5078	27.7468	28	21	31
1	1	62	216.366	234.29	143	1.31183	0.466788	15.4444	0.94843	0.840054	5078	27.7468	28	21	31
1	1	63	216.292	299.953	171	1.47627	0.79437	17.3582	0.94843	0.840054	5078	27.7468	28	21	31
1	1	64	217.121	330.721	171	1.72527	0.81691	11.7586	0.94843	0.840054	5078	27.7468	28	21	31
1	1	65	217.386	427.1	201	1.23232	0.481466	14.3995	0.94843	0.840054	5078	27.7468	28	21	31
1	1	66	222.924	157.769	121	1.22641	0.481466	14.3995	0.94843	0.840054	5078	27.7468	28	21	31
1	1	67	230.071	480.966	435	3.20117	0.749925	23.2442	0.94843	0.840054	5078	27.7468	28	21	31
1	1	68	239.444	265.166	167	1.41008	0.738893	11.3013	0.938202	0.755556	5158	30.8867	32	25	37

EV Table 1.doc

49	243.509	86.7857	224	1.87991	0.816782	16.888	0.899598	0.636344	8827	39.4053	42	30
50	246.831	322.144	160	1.79661	0.80819	16.273	0.91954	0.71555	5025	31.4053	33	31
51	248.234	431.026	71	1.15364	0.49651	9.90149	0.875	0.7	4352	36.3195	39	42
52	255.945	43.616	163	1.58857	0.77002	16.4062	0.91373	0.679167	4720	28.9571	30	32
53	257.061	398.848	66	1.03149	0.243208	9.167	0.916667	0.410015	4440	47.2127	71	50
54	261.492	375.55	251	1.95991	0.860038	17.8769	0.886926	0.597619	10500	41.8227	33	32
55	264.292	273.801	161	1.65004	0.795652	16.3175	0.916773	0.684014	5136	31.9064	32	32
56	264.937	209.402	131	1.31512	0.496472	11.8882	0.917355	0.720719	4858	43.7658	75	46
57	266.137	348.328	131	1.58185	0.774829	12.9149	0.909722	0.652292	9633	73.5344	72	46
58	276.221	171.24	204	2.05613	0.873163	16.1165	0.918519	0.596651	70531	34.5637	35	38
59	277.059	285.089	287	1.78733	0.622935	15.1165	0.872321	0.450191	103520	36.5551	35	42
60	278.327	391.32	150	1.10321	0.422329	13.8198	0.920245	0.765208	9202	41.3167	65	42
61	278.612	391.110	85	1.67318	0.801747	10.4031	0.923913	0.617308	4387	51.4118	55	31
62	285.905	354.719	221	1.56301	0.76855	17.1459	0.931432	0.675139	8580	37.1429	33	41
63	285.326	203.618	231	1.75638	0.822092	16.7746	0.864281	0.701587	10251	46.3846	48	51
64	281.339	355.022	46	1.74017	0.618394	7.65304	0.867923	0.730159	4986	31.67	139	120
65	291.4	319.71	145	1.13164	0.61867	12.5875	0.917722	0.753208	4940	31.049	35	27
66	291.651	442.734	192	2.01531	0.868208	15.6353	0.911176	0.793388	5972	31.1042	32	39
67	293.41	389.276	58	1.25774	0.50651	8.59348	0.920815	0.725	5964	68.3448	70	55
68	299.182	285.162	159	1.39289	0.696112	14.2283	0.929815	0.757143	5103	32.0943	33	26
69	300.14	356.347	150	1.31538	0.649643	13.8198	0.925926	0.78123	5369	35.7933	37	30
70	311.5	260.38	382	1.35137	0.72632	22.054	0.843267	0.598746	16117	42.1911	41	32
71	308.77	332.894	161	1.35063	0.49465	14.3175	0.916047	0.766667	4866	30.4447	33	24
72	307.681	271.811	160	1.62846	0.799304	15.1388	0.932642	0.728745	4915	27.2056	28	22
73	302.483	204.548	126	1.6416	0.791615	12.666	0.9	0.7	4950	29.2857	40	21
74	317.72	43.04	150	2.10013	0.88663	13.8198	0.882353	0.595238	4958	33.0513	35	25
75	315.779	222.448	145	1.2164	0.54542	13.5875	0.917722	0.743559	5048	34.8138	36	28
76	316.432	396.048	147	1.73165	0.878055	17.6809	0.916306	0.773664	8195	62.551	65	47
77	318.371	78.631	213	1.73619	0.688296	17.5897	0.915515	0.755375	8192	31.7119	36	27
78	321.393	171.65	207	1.37961	0.52246	16.2345	0.928251	0.761029	4953	24.1208	25	19
79	327.232	312.526	89	1.72951	0.673818	11.2722	0.932962	0.761538	4632	44.7879	50	35
80	328.495	465.273	153	2.08257	0.746719	13.9573	0.9	0.7	4628	28.9432	30	22
81	327.482	207.401	228	2.08257	0.877601	20.4258	0.896175	0.595703	10208	31.122	33	24
82	335.775	326.084	154	1.36659	0.61577	14.6255	0.928177	0.746667	4848	28.9571	29	23
83	335.775	326.084	154	1.40667	0.73247	13.2023	0.901316	0.713542	4932	50.5985	53	41
84	336.608	376.692	128	1.41662	0.722601	12.8655	0.907718	0.666667	4713	36.7538	38	29
85	340.773	222.659	128	1.82797	0.87097	12.7642	0.920863	0.65441	4609	36.0018	38	29
86	341.524	392.101	149	1.21313	0.566431	13.7736	0.925466	0.764103	4920	33.0201	35	27
87	351.31	211.749	201	1.50456	0.747942	15.9975	0.922018	0.773077	8487	42.2239	43	34
88	342.362	207.665	170	1.49726	0.744283	14.7123	0.909091	0.684259	4955	29.1471	30	23
89	340.316	246.868	152	1.86075	0.84317	13.9116	0.894118	0.596078	5109	33.6118	35	25
90	357.892	316.327	110	1.20428	0.557215	11.8345	0.901639	0.714286	4790	39	41	30
91	361.353	318.483	116	1.51165	0.749822	12.353	0.884596	0.644444	4620	39.8276	42	32
92	361.353	318.483	116	1.73714	0.618512	13.3512	0.933333	0.777778	4854	31.6714	35	27
93	361.353	318.483	116	1.61441	0.61441	11.4518	0.927928	0.792308	4724	45.8441	47	36
94	361.353	318.483	116	2.26284	0.855101	22.0828	0.951311	0.526099	12083	31.5483	30	23
95	371.526	524.683	283	1.35541	0.656322	11.8182	0.908016	0.776224	4428	29.6919	41	31
96	361.353	318.483	116	1.39232	0.39232	15.2444	0.953125	0.871429	4673	25.5355	26	19
97	375.934	102.21	168	1.79676	0.82647	14.6255	0.912013	0.658824	6590	39.2262	41	32
98	377.238	164.469	172	1.55167	0.766024	14.7986	0.910053	0.67451	5008	29.1163	30	24
99	378.103	487.237	150	1.53387	0.68045	15.5536	0.913462	0.694529	6078	31.9895	32	23
100	384.803	129.803	142	1.87278	0.819559	17.1453	0.910391	0.788809	8149	57.3873	61	48
101	381.01	222.932	206	1.51589	0.731547	16.1933	0.927363	0.792308	10014	48.6117	50	37
102	384.354	305.719	96	1.74508	0.819527	11.0538	0.922037	0.666667	4564	47.5117	50	36
103	397.856	400.719	313	1.71338	0.812011	11.9631	0.900512	0.610136	10346	47.6415	46	36
104	389.741	281.944	108	1.86676	0.844417	11.7463	0.892582	0.593037	6866	43.7232	31	53
105	393.748	318.762	105	1.49331	0.742673	11.5624	0.913043	0.830717	4813	43.5933	46	36
106	395.708	20.375	120	1.06414	0.37062	12.3608	0.916031	0.765211	4526	37.2727	39	22
107	402.593	156.493	194	1.87729	0.819903	15.1165	0.926292	0.769841	5702	50.0105	52	45
108	402.562	362.046	130	2.18482	0.893105	12.8655	0.902718	0.640132	4666	35.1231	37	28
109	407.196	35.0714	121	1.34169	0.666695	12.4122	0.909714	0.720238	4622	38.1583	41	31
110	407.174	318.358	109	1.24166	0.610958	11.7806	0.908333	0.658718	4433	40.6697	42	33
111	410.441	432.387	142	2.10237	0.879532	13.4452	0.898736	0.711359	4845	30.165	32	25
112	415.673	271.184	117	1.43507	0.717235	11.4643	0.910955	0.74359	4645	27.4148	32	28
113	418.481	320.456	118	1.36813	0.62468	13.6009	0.93038	0.745625	4576	31.4503	36	31
114	428.737	224.456	172	1.70261	0.809316	12.2573	0.897218	0.605128	4596	38.5192	41	31
115	428.737	36.1016	126	1.24283	0.593793	14.7986	0.92973	0.764444	5120	29.7678	32	24
116	431.477	106.932	222	1.24193	0.593057	12.666	0.926471	0.75	4385	36.3869	35	30
117	431.409	421.282	131	1.74232	0.818951	16.8125	0.925	0.720779	7043	31.7252	32	25
118	431.409	421.282	131	1.70121	0.808953	12.9149	0.923533	0.770388	4751	32.4504	32	25
119	431.409	421.282	131	1.10131	0.62225	12.8159	0.914896	0.767037	4387	35.2718	38	24
120	431.409	421.282	131	1.21593	0.62207	12.666	0.933333	0.75	4445	37.5445	40	30
121	441.29	464.766	124	1.36153	0.621198	12.5631	0.914519	0.68889	4658	37.5445	40	30
122	441.29	152.373	136	1.38303	0.620787	13.159	0.91752	0.700333	5017	36.8897	39	28
123	452.32	495.598	306	1.66314	0.789045	19.7286	0.918919	0.796875	17726	57.9281	61	47
124	451.122	370.49	288	1.77678	0.826562	19.1492	0.925215	0.659039	13388	39.5417	42	39

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
323.098	346.61	373.059	380.573	390.579	401.115	412.284	424.097	436.564	449.684	463.459	477.889	492.974	508.719	525.115	542.152	559.830	578.149	597.209	617.011	637.455	658.542	680.272	702.646	725.664	749.327	773.635	798.589	824.190	850.438
231.301	251.404	271.507	291.610	311.713	331.816	351.919	372.022	392.125	412.228	432.331	452.434	472.537	492.640	512.743	532.846	552.949	573.052	593.155	613.258	633.361	653.464	673.567	693.670	713.773	733.876	753.979	774.082	794.185	814.288
210.337	230.440	250.543	270.646	290.749	310.852	330.955	351.058	371.161	391.264	411.367	431.470	451.573	471.676	491.779	511.882	531.985	552.088	572.191	592.294	612.397	632.500	652.603	672.706	692.809	712.912	733.015	753.118	773.221	793.324
333.916	354.019	374.122	394.225	414.328	434.431	454.534	474.637	494.740	514.843	534.946	555.049	575.152	595.255	615.358	635.461	655.564	675.667	695.770	715.873	735.976	756.079	776.182	796.285	816.388	836.491	856.594	876.697	896.800	916.903
243.415	263.518	283.621	303.724	323.827	343.930	364.033	384.136	404.239	424.342	444.445	464.548	484.651	504.754	524.857	544.960	565.063	585.166	605.269	625.372	645.475	665.578	685.681	705.784	725.887	745.990	766.093	786.196	806.299	826.402
343.518	363.621	383.724	403.827	423.930	444.033	464.136	484.239	504.342	524.445	544.548	564.651	584.754	604.857	624.960	645.063	665.166	685.269	705.372	725.475	745.578	765.681	785.784	805.887	825.990	846.093	866.196	886.299	906.402	926.505
253.518	273.621	293.724	313.827	333.930	354.033	374.136	394.239	414.342	434.445	454.548	474.651	494.754	514.857	534.960	555.063	575.166	595.269	615.372	635.475	655.578	675.681	695.784	715.887	735.990	756.093	776.196	796.299	816.402	836.505
353.621	373.724	393.827	413.930	434.033	454.136	474.239	494.342	514.445	534.548	554.651	574.754	594.857	614.960	635.063	655.166	675.269	695.372	715.475	735.578	755.681	775.784	795.887	815.990	836.093	856.196	876.299	896.402	916.505	936.608
263.621	283.724	303.827	323.930	344.033	364.136	384.239	404.342	424.445	444.548	464.651	484.754	504.857	524.960	545.063	565.166	585.269	605.372	625.475	645.578	665.681	685.784	705.887	725.990	746.093	766.196	786.299	806.402	826.505	846.608
363.724	383.827	403.930	424.033	444.136	464.239	484.342	504.445	524.548	544.651	564.754	584.857	604.960	625.063	645.166	665.269	685.372	705.475	725.578	745.681	765.784	785.887	805.990	826.093	846.196	866.299	886.402	906.505	926.608	946.711
273.724	293.827	313.930	334.033	354.136	374.239	394.342	414.445	434.548	454.651	474.754	494.857	514.960	535.063	555.166	575.269	595.372	615.475	635.578	655.681	675.784	695.887	715.990	736.093	756.196	776.299	796.402	816.505	836.608	856.711
383.827	403.930	424.033	444.136	464.239	484.342	504.445	524.548	544.651	564.754	584.857	604.960	625.063	645.166	665.269	685.372	705.475	725.578	745.681	765.784	785.887	805.990	826.093	846.196	866.299	886.402	906.505	926.608	946.711	966.814
403.930	424.033	444.136	464.239	484.342	504.445	524.548	544.651	564.754	584.857	604.960	625.063	645.166	665.269	685.372	705.475	725.578	745.681	765.784	785.887	805.990	826.093	846.196	866.299	886.402	906.505	926.608	946.711	966.814	986.917
424.033	444.136	464.239	484.342	504.445	524.548	544.651	564.754	584.857	604.960	625.063	645.166	665.269	685.372	705.475	725.578	745.681	765.784	785.887	805.990	826.093	846.196	866.299	886.402	906.505	926.608	946.711	966.814	986.917	1006.920
444.136	464.239	484.342	504.445	524.548	544.651	564.754	584.857	604.960	625.063	645.166	665.269	685.372	705.475	725.578	745.681	765.784	785.887	805.990	826.093	846.196	866.299	886.402	906.505	926.608	946.711	966.814	986.917	1006.920	1026.923
464.239	484.342	504.445	524.548	544.651	564.754	584.857	604.960	625.063	645.166	665.269	685.372	705.475	725.578	745.681	765.784	785.887	805.990	826.093	846.196	866.299	886.402	906.505	926.608	946.711	966.814	986.917	1006.920	1026.923	1046.926
484.342	504.445	524.548	544.651	564.754	584.857	604.960	625.063	645.166	665.269	685.372	705.475	725.578	745.681	765.784	785.887	805.990	826.093	846.196	866.299	886.402	906.505	926.608	946.711	966.814	986.917	1006.920	1026.923	1046.926	1066.929
504.445	524.548	544.651	564.754	584.857	604.960	625.063	645.166	665.269	685.372	705.475	725.578	745.681	765.784	785.887	805.990	826.093	846.196	866.299	886.402	906.505	926.608	946.711	966.814	986.917	1006.920	1026.923	1046.926	1066.929	1086.932
524.548	544.651	564.754	584.857	604.960	625.063	645.166	665.269	685.372	705.475	725.578	745.681	765.784	785.887	805.990	826.093	846.196	866.299	886.402	906.505	926.608	946.711	966.814	986.917	1006.920	1026.923	1046.926	1066.929	1086.932	1106.935
544.651	564.754	584.857	604.960	625.063	645.166	665.269	685.372	705.475	725.578	745.681	765.784	785.887	805.990	826.093	846.196	866.299	886.402	906.505	926.608	946.711	966.814	986.917	1006.920	1026.923	1046.926	1066.929	1086.932	1106.935	1126.938
564.754	584.857	604.960	625.063	645.166	665.269	685.372	705.475	725.578	745.681	765.784	785.887	805.990	826.093	846.196	866.299	886.402	906.505	926.608	946.711	966.814	986.917	1006.920	1026.923	1046.926	1066.929	1086.932	1106.935	1126.938	1146.941
584.857	604.960	625.063	645.166	665.269	685.372	705.475	725.578	745.681	765.784	785.887	805.990	826.093	846.196	866.299	886.402	906.505	926.608	946.711	966.814	986.917	1006.920	1026.923	1046.926	1066.929	1086.932	1106.935	1126.938	1146.941	1166.944
604.960	625.063	645.166	665.269	685.372	705.475	725.578	745.681	765.784	785.887	805.990	826.093	846.196	866.299	886.402	906.505	926.608	946.711	966.814	986.917	1006.920	1026.923	1046.926	1066.929	1086.932	1106.935	1126.938	1146.941	1166.944	1186.947
625.063	645.166	665.269	685.372	705.475	725.578	745.681	765.784	785.887	805.990	826.093	846.196	866.299	886.402	906.505	926.608	946.711	966.814	986.917	1006.920	1026.923	1046.926	1066.929	1086.932	1106.935	1126.938	1146.941	1166.944	1186.947	1206.950
645.166	665.269	685.372	705.475	725.578	745.681	765.784	785.887	805.990	826.093	846.196	866.299	886.402	906.505	926.608	946.711	966.814	986.917	1006.920	1026.923	1046.926	1066.929	1086.932	1106.935	1126.938	1146.941	1166.944	1186.947	1206.950	1226.953
665.269	685.372	705.475	725.578	745.681	765.784	785.887	805.990	826.093	846.196	866.299	886.402	906.505	926.608	946.711	966.814	986.917	1006.920	1026.923	1046.926	1066.929	1086.932	1106.935	1126.938	1146.941	1166.944	1186.947	1206.950	1226.953	1246.956
685.372	705.475	725.578	745.681	765.784	785.887	805.990	826.093	846.196	866.299	886.402	906.505	926.608	946.711	966.814	986.917	1006.920	1026.923	1046.926	1066.929	1086.932	1106.935	1126.938	1146.941	1166.944	1186.947	1206.950	1226.953	1246.956	1266.959
705.475	725.578	745.681	765.784	785.887	805.990	826.093	846.196	866.299	886.402	906.505	926.608	946.711	966.814	986.917	1006.920	1026.923	1046.926	1066.929	1086.932	1106.935	1126.938	1146.941	1166.944	1186.947	1206.950	1226.953	1246.956	1266.959	1286.962
725.578	745.681	765.784	785.887	805.990	826.093	846.196	866.299	886.402	906.505	926.608	946.711	966.814	986.917	1006.920	1026.923	1046.926	1066.929	1086.932	1106.935	1126.938	1146.941	1166.944	1186.947	1206.950	1226.953	1246.956	1266.959	1286.962	1306.965
745.681	765.784	785.887	805.990	826.093	846.196	866.299	886.402	906.505	926.608	946.711	966.814	986.917	1006.920	1026.923	1046.926	1066.929	1086.932	1106.935	1126.938	1146.941	1166.944	1186.947	1206.950	1226.953	1246.956	1266.959	1286.962	1306.965	1326.968
765.784	785.887	805.990	826.093	846.196	866.299	886.402	906.505	926.608	946.711	966.814	986.917	1006.920	1026.923	1046.926	1066.929	1086.932	1106.935	1126.938	1146.941	1166.944	1186.947	1206.950	1226.953	1246.956	1266.959	1286.962	1306.965	1326.968	1346.971
785.887	805.990	826.093	846.196	866.299	886.402	906.505	926.608	946.711	966.814	986.917	1006.920	1026.923	1046.926	1066.929	1086.932	1106.935	1126.938	1146.941	1166.944	1186.947	1206.950	1226.953	1246.956	1266.959	1286.962	1306.965	1326.968	1346.971	1366.974
805.990	826.093	846.196	866.299	886.402	906.505	926.608	946.711	966.814	986.917	1006.920	1026.923	1046.926	1066.929	1086.932	1106.935	1126.938	1146.941	1166.944	1186.947	1206.950	1226.953	1246.956	1266.959	1286.962	1306.965	1326.968			

EV Table 1.doc

1	136	485.08	356.469	299	2.00072	0.93405	19.5115	0.89521	0.69991	1.0053	47.0401	50	38	57
2	137	488.372	106.247	300	1.75179	0.921058	15.5516	0.85856	0.66667	8786	46.2421	50	35	57
3	138	492.318	317.287	155	1.8218	0.913385	15.7371	0.832011	0.71301	5108	27.7133	29	23	34
4	139	500.098	359.008	102	1.21221	0.88118	11.3651	0.81819	0.713281	5508	54	58	39	68
5	140	508.098	119.534	176	1.8992	0.930152	13.4009	0.88034	0.516471	5099	34.4871	37	25	44
6	141	516.517	274.314	176	1.49474	0.914054	14.3956	0.83116	0.617059	8465	56.0569	60	45	68
7	142	523.427	323.427	211	1.20928	0.942286	10.2119	0.83116	0.617059	8465	56.0569	60	45	68
8	143	531.431	374.431	87	1.49712	0.942286	10.2119	0.83116	0.617059	8465	56.0569	60	45	68
9	144	539.431	425.431	71	1.65511	0.913637	9.70466	0.80519	0.703331	9339	55.2031	47	35	55
10	145	547.431	476.431	55	1.81311	0.923029	9.30302	0.80519	0.703331	9339	55.2031	47	35	55
11	146	555.431	527.431	39	2.56212	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
12	147	563.431	578.431	23	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
13	148	571.431	629.431	15	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
14	149	579.431	680.431	7	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
15	150	587.431	731.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
16	151	595.431	782.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
17	152	603.431	833.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
18	153	611.431	884.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
19	154	619.431	935.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
20	155	627.431	986.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
21	156	635.431	1037.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
22	157	643.431	1088.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
23	158	651.431	1139.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
24	159	659.431	1190.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
25	160	667.431	1241.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
26	161	675.431	1292.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
27	162	683.431	1343.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
28	163	691.431	1394.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
29	164	699.431	1445.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
30	165	707.431	1496.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
31	166	715.431	1547.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
32	167	723.431	1598.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
33	168	731.431	1649.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
34	169	739.431	1700.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
35	170	747.431	1751.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
36	171	755.431	1802.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
37	172	763.431	1853.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
38	173	771.431	1904.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
39	174	779.431	1955.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
40	175	787.431	2006.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
41	176	795.431	2057.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
42	177	803.431	2108.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
43	178	811.431	2159.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
44	179	819.431	2210.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
45	180	827.431	2261.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
46	181	835.431	2312.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
47	182	843.431	2363.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
48	183	851.431	2414.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
49	184	859.431	2465.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
50	185	867.431	2516.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
51	186	875.431	2567.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
52	187	883.431	2618.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
53	188	891.431	2669.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
54	189	899.431	2720.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
55	190	907.431	2771.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
56	191	915.431	2822.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
57	192	923.431	2873.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
58	193	931.431	2924.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
59	194	939.431	2975.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
60	195	947.431	3026.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
61	196	955.431	3077.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
62	197	963.431	3128.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
63	198	971.431	3179.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
64	199	979.431	3230.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
65	200	987.431	3281.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
66	201	995.431	3332.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
67	202	1003.431	3383.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
68	203	1011.431	3434.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
69	204	1019.431	3485.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
70	205	1027.431	3536.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
71	206	1035.431	3587.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
72	207	1043.431	3638.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
73	208	1051.431	3689.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
74	209	1059.431	3740.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
75	210	1067.431	3791.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
76	211	1075.431	3842.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
77	212	1083.431	3893.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
78	213	1091.431	3944.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
79	214	1099.431	3995.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983	57.7216	59	44	70
80	215	1107.431	4046.431	1	1.9878	0.920487	13.4161	0.83882	0.59207	3983				

EV Table I.doc

1	3	74	270.921	433.627	177	1.27396	0.624112	15.0121	0.317098	0.713692	97.19	55.0791	35	42	69
2	3	75	272.481	472.443	221	1.76296	0.615964	16.7716	0.618698	0.9272	42.3122	45	33	53	
3	3	76	273.022	545.875	208	2.52522	0.611714	16.2133	0.610497	0.91048	47.1655	45	36	51	
4	3	77	281.51	595.384	488	2.07161	0.617653	16.2387	0.510435	1.3133	51.0102	32	24	78	
5	3	78	283.331	190.639	144	1.96644	0.613353	13.3006	0.5	0.666637	54.3542	56	42	68	
6	3	79	289.363	256.084	219	2.69567	0.620034	17.8035	0.778125	0.523109	42.1134	43	33	51	
7	3	80	290.085	492.403	169	1.23762	0.589177	15.5126	0.521951	0.75	25.0952	27	20	30	
8	3	81	293.268	323.6071	112	1.49137	0.741883	11.9416	0.888889	0.622222	44.2	47	24	39	
9	3	82	286.847	278.133	293	1.2324	0.575125	19.3147	0.531299	0.820728	45.3168	47	38	56	
10	3	83	286.847	44.0877	114	1.52881	0.759372	12.0718	0.890653	0.490909	48.2344	40	31	49	
11	3	84	291.161	204.621	124	1.51011	0.749323	12.5651	0.923194	0.826667	48.4919	51	39	57	
12	3	85	291.161	349.69	129	1.32034	0.659351	12.8159	0.902098	0.716667	48.5116	51	39	58	
13	3	86	299	473.056	234	1.32743	0.657635	17.2609	0.83871	0.724458	56.507	38	28	44	
14	3	87	299.08	329.593	113	1.60716	0.735031	11.9948	0.698823	0.820879	44.708	47	36	54	
15	3	88	305.782	142.492	238	1.37652	0.687198	17.4078	0.923888	0.495906	45	67	35	54	
16	3	89	308.422	376.07	128	1.92602	0.65465	12.7642	0.927536	0.790123	46.6	38	28	45	
17	3	90	310.653	41.0155	373	1.64555	0.796166	20.2795	0.828205	0.821154	50.0155	29	21	42	
18	3	91	317.211	458.408	473	1.49285	0.782186	24.5066	0.852232	0.415845	34.1966	35	25	42	
19	3	92	310.3	259.74	100	1.6277	0.786163	11.2878	0.909091	0.714786	48.75	51	38	58	
20	3	93	314.394	172.431	109	1.70657	0.810132	11.7806	0.938655	0.407607	42.9541	46	34	54	
21	3	94	319.03	158.242	95	1.44078	0.719907	11.2272	0.908237	0.692308	47.199	69	39	58	
22	3	95	320.597	216.664	134	1.40176	0.700768	13.0619	0.930556	0.812121	31.6791	37	28	46	
23	3	96	330.547	247.988	139	1.17321	0.523072	13.3034	0.926687	0.163736	36.0432	36	24	46	
24	3	97	337.379	327.823	271	2.09271	0.878442	18.3755	0.808955	0.6775	37.631	40	29	46	
25	3	98	333.177	395.24	192	1.71739	0.81299	15.4353	0.667728	0.627651	53.2512	54	41	66	
26	3	99	331.564	104.017	172	1.46903	0.783419	14.7966	0.924721	0.151786	57.8023	60	48	69	
27	3	100	338.308	360.632	133	1.15592	0.501454	13.0131	0.923611	0.791667	39.406	40	31	47	
28	3	101	342.452	380.018	124	1.40324	0.701558	12.5631	0.918519	0.881318	42.0565	65	33	51	
29	3	102	351.184	235.709	179	1.78723	0.826814	15.0967	0.939497	0.612377	28.1687	29	22	35	
30	3	103	349.87	267.407	161	1.21395	0.566948	14.3175	0.947059	0.825641	31.7143	32	24	38	
31	3	104	355.573	192.699	206	1.75463	0.8117	16.1953	0.919643	0.44375	37.8447	38	29	47	
32	3	105	362.143	126.473	357	1.5789	0.771864	11.3701	0.919643	0.686538	37.8447	38	29	47	
33	3	106	365.192	19.6384	177	1.28705	0.695539	15.0121	0.931579	0.761667	54.2913	57	40	67	
34	3	107	364.781	79.4298	114	1.04965	0.341947	12.0718	0.912	0.730769	27.209	29	21	33	
35	3	108	364.542	350.4	120	1.4671	0.722819	12.3408	0.944882	0.8	38.075	60	32	46	
36	3	109	368.913	285.978	166	1.36168	0.619895	17.65304	0.884615	0.638889	117.522	151	117	181	
37	3	110	380.866	338.11	127	1.10246	0.421706	12.7162	0.913659	0.151479	37.6162	40	30	46	
38	3	111	381.023	50.5196	131	1.57163	0.76328	12.9169	0.916094	0.74378	32.3571	34	27	40	
39	3	112	384.055	201.742	165	1.49253	0.74236	14.4943	0.953757	0.808824	59.4061	62	48	70	
40	3	113	384.368	104.735	195	2.07424	0.816514	15.757	0.933014	0.770751	26.3716	28	19	33	
41	3	114	398.613	381.047	134	1.35801	0.676536	13.0519	0.937063	0.812131	36.7164	39	30	43	
42	3	115	402.104	171.906	202	1.96693	0.861118	16.0779	0.918182	0.655844	27.104	28	22	33	
43	3	116	402.82	214.787	239	1.7078	0.810637	17.4433	0.895131	0.661869	37.8954	58	43	71	
44	3	117	401.701	469.348	117	1.49292	0.742919	12.2053	0.936	0.75974	37.2735	39	29	46	
45	3	118	412.597	12.0269	167	2.25991	0.896771	15.389	0.920792	0.673913	25.2688	27	19	31	
46	3	119	420.631	404.34	137	1.87173	0.845318	13.2073	0.913333	0.652381	44.9854	46	36	54	
47	3	120	421.21	438.29	100	1.74133	0.816664	11.2818	0.846556	0.666667	41.25	42	32	51	
48	3	121	421.597	210.442	67	1.71433	0.616053	9.23618	0.846556	0.666667	59.8209	60	47	71	
49	3	122	428.803	126.659	132	1.33134	0.684287	12.9416	0.917808	0.697917	34.7727	35	27	43	
50	3	123	438.479	448.696	112	1.25339	0.67329	11.9416	0.92562	0.8	40.2368	42	33	48	
51	3	124	440.679	30.7668	193	1.43016	0.813524	15.6758	0.92562	0.8	41.0725	25	19	29	
52	3	125	441.716	399.413	246	1.38419	0.696963	17.6978	0.919048	0.70695	24.3134	30	22	35	
53	3	126	439.703	203.993	75	1.92194	0.83298	9.77205	0.935241	0.719298	57.2267	61	44	71	
54	3	127	439.308	324.977	44	1.33865	0.644673	7.48482	0.920213	0.783714	155.977	161	120	193	
55	3	128	441.302	138.214	173	1.15723	0.503268	14.8415	0.920213	0.720833	28.5434	30	22	35	
56	3	129	445.371	448.111	95	1.25659	0.605631	10.8981	0.954522	0.783714	165.118	176	108	193	
57	3	130	441.353	181.412	34	1.21165	0.592769	8.57952	0.95	0.708333	35.4077	38	28	42	
58	3	131	447.042	79.8769	130	1.11537	0.423558	12.6552	0.926571	0.77381	35.2105	36	27	42	
59	3	132	449.534	70.0451	133	1.03711	0.318099	15.0321	0.907423	0.676531	42.2268	42	37	51	
60	3	133	450.028	356.36	114	1.20305	0.827186	12.0718	0.941118	0.74026	38.7218	31	24	42	
61	3	134	460.248	100.075	224	1.20305	0.924259	14.9423	0.846782	0.788899	50.5915	60	48	70	
62	3	135	451.638	478.534	71	1.27837	0.621423	5.30185	0.910258	0.758978	37.1757	38	29	44	
63	3	136	461.959	250.088	148	1.36267	0.679488	13.7273	0.936709	0.758978	114.322	118	87	134	
64	3	137	466.043	56.1739	23	1.31472	0.693198	5.01152	0.951852	0.457143	121.5	132	87	134	
65	3	138	465.3	63.85	20	1.20213	0.555313	5.04627	0.933332	0.666667	62.107	66	51	77	
66	3	139	474.931	370.517	87	1.37483	0.666253	10.5248	0.918788	0.660231	63.1379	66	51	77	
67	3	140	476.665	164.937	158	1.35123	0.655422	14.1835	0.924612	0.774531	53.3038	56	42	70	
68	3	141	475.295	107.409	64	1.52203	0.733875	7.48482	0.916667	0.698413	152.888	156	111	188	
69	3	142	482.725	427.554	204	1.77691	0.826609	16.1165	0.916667	0.698413	62.093	62	47	72	
70	3	143	485.122	58.083	294	1.44407	0.721431	19.3777	0.868822	0.786096	132.755	69	35	59	
71	3	144	482.755	451.941	102	1.96739	0.838085	11.3961	0.907455	0.708333	60.893	62	47	72	
72	3	145	480.108	493.43	120	1.77474	0.861646	12.3608	0.907455	0.708333	35.463	36	27	44	
73	3	146	482.899	14.649	158	1.2289	0.581235	14.1835	0.916667	0.708333					

EV Table 1.doc

1	1	491.486	286.016	35	1.14928	0.541385	6.47558	0.545916	0.433333	3545	101.288	105	89	117
2	1	491.438	286.016	130	1.20206	0.471891	12.4655	0.502778	0.433333	4553	35.0231	36	26	43
3	1	499.436	144.508	128	1.24005	0.531851	12.1642	0.520843	0.711111	4856	37.9375	39	30	46
4	1	504.436	285.462	39	1.4304	0.715017	12.0472	0.906971	0.8125	3880	94.359	96	70	113
5	1	20	122.471	152	1.3561	0.475449	12.9116	0.521212	0.710168	4832	31.7895	33	25	39
6	1	22.2001	122.3198	197	1.33473	0.462327	12.4376	0.529245	0.72953	9490	48.1726	50	37	59
7	1	22.1391	140.043	115	1.35777	0.461433	12.1003	0.521933	0.405842	4511	39.2261	40	30	48
8	1	23.2174	287.022	137	1.68449	0.604492	12.2013	0.529439	0.490714	7260	44.1022	47	35	53
9	1	26.6026	344.243	151	1.68449	0.604492	12.2013	0.529439	0.490714	7260	44.1022	47	35	53
10	1	26.0394	162.559	102	1.28299	0.621493	11.3561	0.53578	0.784635	4532	48.0795	50	36	57
11	1	26.734	487.684	282	1.28299	0.621493	11.3561	0.53578	0.784635	4532	48.0795	50	36	57
12	1	39.2551	188.777	183	1.28299	0.621493	11.3561	0.53578	0.784635	4532	48.0795	50	36	57
13	1	49.5199	217.316	377	2.50967	0.930606	21.9052	0.821351	0.419335	13413	44.1022	47	35	53
14	1	40.3109	306.084	119	1.46633	0.731315	12.3092	0.529439	0.490714	7260	44.1022	47	35	53
15	1	49.8124	124.27	259	2.13833	0.883911	18.1555	0.793933	0.535121	10590	38.4739	41	29	48
16	1	53.6559	76.3118	166	1.22662	0.579108	15.389	0.544142	0.781513	9169	50.3068	53	42	60
17	1	61.75	496.487	156	1.3807	0.695518	14.0925	0.524571	0.714706	6041	41.0221	42	29	51
18	1	61.8138	110.813	96	1.5615	0.769055	11.0559	0.597196	0.671328	4500	46.475	48	38	58
19	1	70.4153	190.465	172	2.30563	0.90107	14.7986	0.900524	0.532508	5938	33.9419	35	27	41
20	1	69.6514	97.7718	81	1.41608	0.717112	10.1354	0.5	0.735	4131	54.7037	58	43	65
21	1	87.8529	100.461	102	1.86101	0.843364	11.3561	0.594737	0.706333	4364	42.7843	46	33	52
22	1	89.9759	68.9148	100	1.14834	0.61535	11.7265	0.515254	0.755245	4397	60.4701	63	32	46
23	1	98.8146	103.474	190	2.31245	0.901662	15.5536	0.92233	0.488406	7736	40.7158	42	32	46
24	1	98.8018	253.413	111	1.39113	0.655175	12.8882	0.533086	0.747028	10787	42.8761	46	34	51
25	1	96.4018	405.521	221	1.31667	0.605287	12.8882	0.517355	0.74	4686	42.2162	44	33	51
26	1	98.7228	41.5218	101	1.22405	0.648287	12.4122	0.516667	0.785716	4595	37.9752	41	29	46
27	1	100.188	110.136	176	1.48194	0.718011	14.9696	0.926316	0.735096	5059	42.8218	44	33	52
28	1	101.765	300.07	115	1.86325	0.813776	12.1005	0.884615	0.589746	4560	39.4522	41	31	49
29	1	108.123	141.793	135	1.04071	0.333452	13.1106	0.518347	0.711358	4866	35.0144	36	28	45
30	1	118.775	642.208	18	2.78411	0.895059	7.41764	0.88189	0.622222	4387	33.1339	41	31	47
31	1	124.705	39.2679	112	1.93296	0.769275	11.9416	0.92437	0.705128	4488	40.8	43	33	49
32	1	132.518	319.373	110	1.56515	0.560153	12.7662	0.807601	0.741905	4594	34.6719	37	29	45
33	1	130.461	400.248	125	1.20859	0.676922	12.6157	0.925928	0.757576	4636	37.088	38	28	45
34	1	135.846	109.592	182	1.19873	0.531436	15.2229	0.924571	0.764706	5123	28.1404	29	22	34
35	1	131.5	264.779	104	1.25554	0.603338	14.4503	0.921318	0.759259	4936	30.2195	31	24	36
36	1	131.316	644.402	128	1.25554	0.603338	14.4503	0.921318	0.759259	4936	30.2195	31	24	36
37	1	134.318	471.929	157	1.45981	0.788134	14.1386	0.926991	0.641467	4848	35.7031	39	30	46
38	1	137.259	431.776	116	1.53578	0.758963	13.3512	0.915033	0.729147	4828	39.181	38	29	49
39	1	137.419	292.532	160	1.03316	0.232051	14.0935	0.921077	0.712657	9109	34.4857	35	27	47
40	1	160.534	163.142	156	1.71923	0.813434	12.4122	0.896286	0.664035	4553	37.4201	39	29	49
41	1	164.635	45.5079	43	1.72672	0.815232	8.95623	0.913043	0.63	7016	111.265	116	91	136
42	1	168.561	321.11	82	1.6395	0.792517	10.2179	0.87234	0.621212	4075	49.4951	52	40	60
43	1	167.266	389.735	98	1.6395	0.792517	10.2179	0.87234	0.621212	4075	49.4951	52	40	60
44	1	171.548	93.6129	311	2.5791	0.921772	20.8369	0.933741	0.645833	18949	43.8387	45	33	55
45	1	170.747	210.378	61	1.21347	0.56864	10.5348	0.915789	0.750909	4778	54.9195	56	43	65
46	1	172.538	392.157	170	1.4118	0.72038	12.8635	0.915493	0.714286	4816	37.0462	37	29	46
47	1	187.753	453.167	194	1.03532	0.236937	10.1554	0.91166	0.81	4816	59.4568	60	45	71
48	1	187.753	453.167	194	1.03532	0.236937	10.1554	0.91166	0.81	4816	59.4568	60	45	71
49	1	207.142	275.591	154	1.57599	0.722905	17.1515	0.911748	0.714648	5054	24.0515	27	20	32
50	1	208.346	152.4718	112	1.34656	0.609816	14.0028	0.933333	0.802083	7816	41.4591	42	31	53
51	1	208.346	152.4718	112	1.34656	0.609816	14.0028	0.933333	0.802083	7816	41.4591	42	31	53
52	1	214.56	292.421	254	1.62551	0.801815	17.9034	0.922078	0.735583	4162	48.1558	51	37	62
53	1	214.56	292.421	254	1.62551	0.801815	17.9034	0.922078	0.735583	4162	48.1558	51	37	62
54	1	221.36	332.114	125	1.10482	0.425319	12.6157	0.911111	0.732143	7199	60.9575	63	51	73
55	1	221.36	332.114	125	1.10482	0.425319	12.6157	0.911111	0.732143	7199	60.9575	63	51	73
56	1	221.36	332.114	125	1.10482	0.425319	12.6157	0.911111	0.732143	7199	60.9575	63	51	73
57	1	230.372	402.352	128	1.20282	0.565289	12.7462	0.907801	0.65691	5212	40.7188	41	31	50
58	1	236.016	19.3032	167	1.40843	0.701152	14.5819	0.925453	0.755556	4581	27.4311	29	22	34
59	1	238.949	345.441	117	1.271	0.617231	12.2853	0.926571	0.739774	4816	39.453	41	30	48
60	1	241.722	275.591	154	1.37505	0.684318	10.1554	0.95011	0.75	6038	74.5127	78	59	87
61	1	242.159	247.41	385	2.41941	0.940375	22.1404	0.97819	0.714697	4235	29.4803	31	23	36
62	1	259.684	356.2	95	1.26027	0.507228	20.5991	0.98745	0.714697	4235	29.4803	31	23	36
63	1	246.622	143.438	315	1.83148	0.737859	10.0247	0.981457	0.711111	8149	24.1758	34	26	57
64	1	246.622	143.438	315	1.83148	0.737859	10.0247	0.981457	0.711111	8149	24.1758	34	26	57
65	1	271.555	246.079	256	1.57033	0.711011	10.0541	0.981457	0.711011	8149	24.1758	34	26	57
66	1	271.555	246.079	256	1.57033	0.711011	10.0541	0.981457	0.711011	8149	24.1758	34	26	57
67	1	273.004	327.134	97	1.97723	0.751639	15.3177	0.925581	0.69972	4521	22.7186	24	18	27
68	1	278.578	102.532	109	1.66155	0.746166	11.1232	0.923774	0.723944	4365	49.5872	50	37	63
69	1	279.534	305.402	103	1.37098	0.881081	11.4518	0.884179	0.82364	5105	42.8252	44	32	52
70	1	289.613	488.525	261	2.59776	0.943721	18.2295	0.952931	0.762024	4411	32.8207	36	27	46
71	1	290.379	451.764	55	1.69525	0.9085	8.38828	0.952016	0.611111	5053	115.416	120	91	162
72	1	290.379	451.764	55	1.69525	0.9085	8.38828	0.952016	0.611111	5053	115.416	120	91	162
73	1	290.379	451.764	55	1.69525	0.9085	8.38828	0.952016	0.611111	5053	115.416	120	91	162

EV Table 1.doc

1	308.012	29.431	170	1.2847	0.62778	14.7123	0.505091	0.574603	4598	27.0171	28	21	33
2	306.881	27.524	126	1.37001	0.66166	12.566	0.593617	0.5	4480	35.5556	38	28	43
3	306.739	25.899	126	1.47498	0.714498	9.5115	0.611585	0.479545	20353	68.0702	71	55	81
4	306.599	24.274	126	1.57218	0.768264	18.4724	0.631214	0.420317	9821	34.6455	39	27	46
5	306.459	22.649	126	1.67045	0.821437	12.0092	0.648478	0.372222	7187	43.7692	46	35	52
6	306.319	21.024	126	1.76872	0.874610	12.0092	0.665688	0.324444	4678	39.3109	41	29	48
7	306.179	19.399	126	1.86699	0.927781	12.0092	0.682898	0.276666	2141	31.6016	39	30	44
8	306.039	17.774	126	1.96526	0.980952	12.0092	0.700108	0.228888	625	27.2626	45	32	53
9	305.899	16.149	126	2.06353	1.034123	12.0092	0.717324	0.181111	15208	29.4592	31	23	38
10	305.759	14.524	126	2.16180	1.087294	12.0092	0.734540	0.133333	3726	37.0875	36	29	45
11	305.619	12.899	126	2.26007	1.140465	12.0092	0.751756	0.085555	5405	35.2917	35	26	41
12	305.479	11.274	126	2.35834	1.193636	12.0092	0.768972	0.037777	4717	34.1812	35	26	41
13	305.339	9.649	126	2.45661	1.246807	12.0092	0.786188	0.000000	4451	32.9531	41	31	47
14	305.199	8.024	126	2.55488	1.299978	12.0092	0.803404	0.000000	8924	31.7250	43	32	51
15	305.059	6.399	126	2.65315	1.353149	12.0092	0.820620	0.000000	8924	30.4969	33	24	38
16	304.919	4.774	126	2.75142	1.406320	12.0092	0.837836	0.000000	7926	29.2688	31	23	35
17	304.779	3.149	126	2.84969	1.459491	12.0092	0.855052	0.000000	5314	28.0407	36	29	45
18	304.639	1.524	126	2.94796	1.512662	12.0092	0.872268	0.000000	4717	26.8126	35	26	41
19	304.499	0.000	126	3.04623	1.565833	12.0092	0.889484	0.000000	4451	25.5845	31	23	35
20	304.359	0.000	126	3.14450	1.619004	12.0092	0.906700	0.000000	4185	24.3564	30	20	34
21	304.219	0.000	126	3.24277	1.672175	12.0092	0.923916	0.000000	3919	23.1283	29	19	33
22	304.079	0.000	126	3.34104	1.725346	12.0092	0.941132	0.000000	3653	21.9002	28	18	32
23	303.939	0.000	126	3.43931	1.778517	12.0092	0.958348	0.000000	3387	20.6721	27	17	31
24	303.799	0.000	126	3.53758	1.831688	12.0092	0.975564	0.000000	3121	19.4440	26	16	30
25	303.659	0.000	126	3.63585	1.884859	12.0092	0.992780	0.000000	2855	18.2159	25	15	29
26	303.519	0.000	126	3.73412	1.938030	12.0092	1.010000	0.000000	2589	16.9878	24	14	28
27	303.379	0.000	126	3.83239	1.991201	12.0092	1.027222	0.000000	2323	15.7597	23	13	27
28	303.239	0.000	126	3.93066	2.044372	12.0092	1.044444	0.000000	2057	14.5316	22	12	26
29	303.099	0.000	126	4.02893	2.097543	12.0092	1.061666	0.000000	1791	13.3035	21	11	25
30	302.959	0.000	126	4.12720	2.150714	12.0092	1.078888	0.000000	1525	12.0754	20	10	24
31	302.819	0.000	126	4.22547	2.203885	12.0092	1.096111	0.000000	1259	10.8473	19	9	23
32	302.679	0.000	126	4.32374	2.257056	12.0092	1.113333	0.000000	993	9.6192	18	8	22
33	302.539	0.000	126	4.42201	2.310227	12.0092	1.130555	0.000000	727	8.3911	17	7	21
34	302.399	0.000	126	4.52028	2.363398	12.0092	1.147777	0.000000	461	7.1630	16	6	20
35	302.259	0.000	126	4.61855	2.416569	12.0092	1.165000	0.000000	195	5.9349	15	5	19
36	302.119	0.000	126	4.71682	2.469740	12.0092	1.182222	0.000000	0	4.7068	14	4	18
37	301.979	0.000	126	4.81509	2.522911	12.0092	1.199444	0.000000	0	3.4787	13	3	17
38	301.839	0.000	126	4.91336	2.576082	12.0092	1.216666	0.000000	0	2.2506	12	2	16
39	301.699	0.000	126	5.01163	2.629253	12.0092	1.233888	0.000000	0	1.0225	11	1	15
40	301.559	0.000	126	5.10990	2.682424	12.0092	1.251111	0.000000	0	0.0000	10	0	14
41	301.419	0.000	126	5.20817	2.735595	12.0092	1.268333	0.000000	0	0.0000	9	0	13
42	301.279	0.000	126	5.30644	2.788766	12.0092	1.285555	0.000000	0	0.0000	8	0	12
43	301.139	0.000	126	5.40471	2.841937	12.0092	1.302777	0.000000	0	0.0000	7	0	11
44	300.999	0.000	126	5.50298	2.895108	12.0092	1.320000	0.000000	0	0.0000	6	0	10
45	300.859	0.000	126	5.60125	2.948279	12.0092	1.337222	0.000000	0	0.0000	5	0	9
46	300.719	0.000	126	5.69952	3.001450	12.0092	1.354444	0.000000	0	0.0000	4	0	8
47	300.579	0.000	126	5.79779	3.054621	12.0092	1.371666	0.000000	0	0.0000	3	0	7
48	300.439	0.000	126	5.89606	3.107792	12.0092	1.388888	0.000000	0	0.0000	2	0	6
49	300.299	0.000	126	5.99433	3.160963	12.0092	1.406111	0.000000	0	0.0000	1	0	5
50	300.159	0.000	126	6.09260	3.214134	12.0092	1.423333	0.000000	0	0.0000	0	0	4
51	300.019	0.000	126	6.19087	3.267305	12.0092	1.440555	0.000000	0	0.0000	0	0	3
52	299.879	0.000	126	6.28914	3.320476	12.0092	1.457777	0.000000	0	0.0000	0	0	2
53	299.739	0.000	126	6.38741	3.373647	12.0092	1.475000	0.000000	0	0.0000	0	0	1
54	299.599	0.000	126	6.48568	3.426818	12.0092	1.492222	0.000000	0	0.0000	0	0	0
55	299.459	0.000	126	6.58395	3.480000	12.0092	1.509444	0.000000	0	0.0000	0	0	0
56	299.319	0.000	126	6.68222	3.533171	12.0092	1.526666	0.000000	0	0.0000	0	0	0
57	299.179	0.000	126	6.78049	3.586342	12.0092	1.543888	0.000000	0	0.0000	0	0	0
58	299.039	0.000	126	6.87876	3.639513	12.0092	1.561111	0.000000	0	0.0000	0	0	0
59	298.899	0.000	126	6.97703	3.692684	12.0092	1.578333	0.000000	0	0.0000	0	0	0
60	298.759	0.000	126	7.07530	3.745855	12.0092	1.595555	0.000000	0	0.0000	0	0	0
61	298.619	0.000	126	7.17357	3.799026	12.0092	1.612777	0.000000	0	0.0000	0	0	0
62	298.479	0.000	126	7.27184	3.852197	12.0092	1.630000	0.000000	0	0.0000	0	0	0
63	298.339	0.000	126	7.37011	3.905368	12.0092	1.647222	0.000000	0	0.0000	0	0	0
64	298.199	0.000	126	7.46838	3.958539	12.0092	1.664444	0.000000	0	0.0000	0	0	0
65	298.059	0.000	126	7.56665	4.011710	12.0092	1.681666	0.000000	0	0.0000	0	0	0
66	297.919	0.000	126	7.66492	4.064881	12.0092	1.698888	0.000000	0	0.0000	0	0	0
67	297.779	0.000	126	7.76319	4.118052	12.0092	1.716111	0.000000	0	0.0000	0	0	0
68	297.639	0.000	126	7.86146	4.171223	12.0092	1.733333	0.000000	0	0.0000	0	0	0
69	297.499	0.000	126	7.95973	4.224394	12.0092	1.750555	0.000000	0	0.0000	0	0	0
70	297.359	0.000	126	8.05800	4.277565	12.0092	1.767777	0.000000	0	0.0000	0	0	0
71	297.219	0.000	126	8.15627	4.330736	12.0092	1.785000	0.000000	0	0.0000	0	0	0
72	297.079	0.000	126	8.25454	4.383907	12.0092	1.802222	0.000000	0	0.0000	0	0	0
73	296.939	0.000	126	8.35281	4.437078	12.0092	1.819444	0.000000	0	0.0000	0	0	0
74	296.799	0.000	126	8.45108	4.490249	12.0092	1.836666	0.000000	0	0.0000	0	0	0
75	296.659	0.000	126	8.54935	4.543420	12.0092	1.853888	0.000000	0	0.0000	0	0	0
76	296.519	0.000	126	8.64762	4.596591	12.0092	1.871111	0.000000	0	0.0000	0	0	0
77	296.379	0.000	126	8.74589	4.649762	12.0092	1.888333	0.000000	0	0.0000	0	0	0
78	296.239	0.000	126	8.84416	4.702933	12.0092	1.905555	0.000000	0	0.0000	0	0	0
79	296.099	0.000	126	8.94243	4.756104	12.0092	1.922777	0.000000	0	0.0000	0	0	0
80	295.959	0.000	126	9.04070	4.809275	12.0092	1.940000	0.000000	0	0.0000	0	0	0
81	295.819	0.000	126	9.13897	4.862446	12.0092	1.957222	0.000000	0	0.0000	0	0	0
82	295.679	0.000	126	9.23724	4.915617	12.0092	1.974444	0.000000	0	0.0000	0	0	0
83	295.539	0.000	126	9.33551	4.968788	12.0092	1.991666	0.000000	0	0.0000	0	0	0
84	295.399	0.000	126	9.43378	5.021959	12.0092	2.008888	0.000000	0	0.0000	0	0	0
85	295.259	0.000	126	9.53205	5.075130	12.0092	2.026111	0.000000	0	0.0000	0	0	0
86	295.119	0.000	126	9.63032	5.128301	12.0092	2.043333	0.0000					

EV Table 1.doc	1	5	36	244.003	44.7166	334	1.70565	0.010104	20.6219	0.4895942	0.702158	15584	49	38	55		
	1	5	37	246.434	115.89	145	1.57559	0.717771	13.5075	0.917772	0.497113	4889	32	25	41		
	1	5	38	245.163	344.532	173	1.4535	0.725714	14.6115	0.903759	0.490785	9592	51	31	46		
	1	5	39	259.465	297.804	181	1.74551	0.919677	13.7273	0.907975	0.465483	6156	41	4041	41		
	1	5	40	255.892	35.5	186	1.69725	0.805796	15.3893	0.925373	0.465333	7114	41	421	41		
	1	5	41	267.611	474.88	288	1.69734	0.409018	19.1492	0.857143	0.426087	17451	43	2326	43		
	1	5	42	276.033	256.171	152	1.66024	0.758253	13.9116	0.91018	0.703704	6365	41	875	41		
	1	5	43	268.878	481.744	133	1.6018	0.421848	12.5163	0.917991	0.405222	4486	38	7025	38		
	1	5	44	295.075	427.753	116	1.6956	0.405009	12.6163	0.918233	0.717773	5724	39	7025	39		
	1	5	45	318.666	179.541	322	1.79398	0.930231	12.4634	0.917293	0.797186	6081	38	7025	38		
	1	5	46	310.664	171.228	254	1.95525	0.959316	17.9434	0.910065	0.574661	7079	38	4528	41		
	1	5	47	321.634	410.756	331	1.76161	0.621266	32.9149	0.897478	0.468387	18781	63	43	71		
	1	5	48	331.614	418.455	391	1.46651	0.732338	22.3123	0.807851	0.258394	18781	47	9308	47		
	1	5	49	339.339	87.0639	143	1.66512	0.847704	13.4935	0.910628	0.752512	4651	37	3385	34		
	1	5	50	346.493	100.533	219	2.21367	0.892757	16.4995	0.910286	0.758364	4167	35	7123	24		
	1	5	51	348.326	442.7	174	1.41208	0.760634	14.8443	0.935464	0.728401	5191	41	22	335	24	
	1	5	52	356.336	136.177	232	1.8831	0.448052	17.187	0.939711	0.752001	9139	41	22	335	24	
	1	5	53	363.332	176.751	265	1.64527	0.793621	15.3657	0.868628	0.752001	9139	41	22	335	24	
	1	5	54	368.601	52.3114	288	1.73712	0.817688	17.0382	0.868628	0.752001	9139	41	22	335	24	
	1	5	55	372.372	179.938	212	1.79951	0.705149	16.4482	0.938326	0.717232	9139	41	22	335	24	
	1	5	56	374.725	117.607	150	1.93951	0.494272	32.9116	0.912112	0.731775	4477	37	3313	34		
	1	5	57	374.203	371.178	152	1.55456	0.721618	12.1005	0.912112	0.731775	4477	37	3313	34		
	1	5	58	377.48	376.532	115	1.45456	0.841296	16.4033	0.909512	0.731775	4477	37	3313	34		
	1	5	59	378.391	363.124	266	1.64987	0.873671	17.1127	0.918325	0.731775	4477	37	3313	34		
	1	5	60	384.356	268.113	230	2.04718	0.873671	17.1127	0.918325	0.731775	4477	37	3313	34		
	1	5	61	385.282	492.812	202	1.66295	0.847372	16.0373	0.905185	0.751512	8567	37	1541	39	41	
	1	5	62	410.504	459.777	193	1.74583	0.420613	11.3034	0.908197	0.462054	4291	30	4705	32	31	36
	1	5	63	422.454	466.6	259	1.41752	0.708732	18.1595	0.945555	0.708033	5626	37	166	37	166	37
	1	5	64	422.454	175.104	238	2.81888	0.874505	20.4358	0.815592	0.595574	10429	31	7957	31	75	42
	1	5	65	440.31	90.8832	203	1.61411	0.764264	16.2003	0.828844	0.613913	12182	31	3059	31	55	45
	1	5	66	440.31	375.656	251	1.37159	0.664118	16.0773	0.937778	0.701369	9071	41	4584	41	77	54
	1	5	67	454.735	375.656	251	2.37619	0.911862	16.0093	0.925517	0.708033	4291	30	4705	30	41	68
	1	5	68	452.566	164.485	316	1.59197	0.772014	13.1353	0.935312	0.608466	13405	37	2389	37	40	63
	1	5	69	468.418	235.218	284	1.32695	0.657335	35.3177	0.930508	0.608466	13405	37	2389	37	40	63
	1	5	70	468.418	264.417	326	1.6291	0.789332	30.7374	0.915372	0.463317	8203	35	2655	35	46	66
	1	5	71	465.012	47.3256	96	1.92317	0.855575	33.0558	0.90554	0.463317	8203	35	2655	35	46	66
	1	5	72	471.132	211.108	251	1.78261	0.827832	17.6169	0.929234	0.730214	4120	37	1171	37	40	45
	1	5	73	466.162	66.9279	111	1.23208	0.365076	11.8882	0.905816	0.463317	8203	35	2655	35	46	66
	1	5	74	472.917	140.062	168	1.49961	0.765199	14.4255	0.908016	0.718719	5593	30	1507	32	25	41
	1	5	75	472.917	140.062	168	1.49961	0.765199	14.4255	0.908016	0.718719	5593	30	1507	32	25	41
	1	5	76	472.917	140.062	168	1.49961	0.765199	14.4255	0.908016	0.718719	5593	30	1507	32	25	41
	1	5	77	480.308	181.804	159	1.49961	0.765199	14.4255	0.908016	0.718719	5593	30	1507	32	25	41
	1	5	78	480.308	181.804	159	1.49961	0.765199	14.4255	0.908016	0.718719	5593	30	1507	32	25	41
	1	5	79	480.308	181.804	159	1.49961	0.765199	14.4255	0.908016	0.718719	5593	30	1507	32	25	41
	1	5	80	480.308	181.804	159	1.49961	0.765199	14.4255	0.908016	0.718719	5593	30	1507	32	25	41
	1	5	81	492.335	466.939	124	1.49235	0.492335	466.939	0.914063	0.920208	3922	33	5214	34	26	41
	1	5	82	500.468	432.352	142	1.24025	0.617413	12.1551	0.914063	0.920208	3922	33	5214	34	26	41
	1	5	83	500.468	432.352	142	1.24025	0.617413	12.1551	0.914063	0.920208	3922	33	5214	34	26	41
	1	5	84	500.468	432.352	142	1.24025	0.617413	12.1551	0.914063	0.920208	3922	33	5214	34	26	41
	1	5	85	500.468	432.352	142	1.24025	0.617413	12.1551	0.914063	0.920208	3922	33	5214	34	26	41
	1	5	86	500.468	432.352	142	1.24025	0.617413	12.1551	0.914063	0.920208	3922	33	5214	34	26	41
	1	5	87	500.468	432.352	142	1.24025	0.617413	12.1551	0.914063	0.920208	3922	33	5214	34	26	41
	1	5	88	500.468	432.352	142	1.24025	0.617413	12.1551	0.914063	0.920208	3922	33	5214	34	26	41
	1	5	89	500.468	432.352	142	1.24025	0.617413	12.1551	0.914063	0.920208	3922	33	5214	34	26	41
	1	5	90	500.468	432.352	142	1.24025	0.617413	12.1551	0.914063	0.920208	3922	33	5214	34	26	41
	1	5	91	500.468	432.352	142	1.24025	0.617413	12.1551	0.914063	0.920208	3922	33	5214	34	26	41
	1	5	92	500.468	432.352	142	1.24025	0.617413	12.1551	0.914063	0.920208	3922	33	5214	34	26	41
	1	5	93	500.468	432.352	142	1.24025	0.617413	12.1551	0.914063	0.920208	3922	33	5214	34	26	41
	1	5	94	500.468	432.352	142	1.24025	0.617413	12.1551	0.914063	0.920208	3922	33	5214	34	26	41
	1	5	95	500.468	432.352	142	1.24025	0.617413	12.1551	0.914063	0.920208	3922	33	5214	34	26	41
	1	5	96	500.468	432.352	142	1.24025	0.617413	12.1551	0.914063	0.920208	3922	33	5214	34	26	41
	1	5	97	500.468	432.352	142	1.24025	0.617413	12.1551	0.914063	0.920208	3922	33	5214	34	26	41
	1	5	98	500.468	432.352	142	1.24025	0.617413	12.1551	0.914063	0.920208	3922	33	5214	34	26	41
	1	5	99	500.468	432.352	142	1.24025	0.617413	12.1551	0.914063	0.920208	3922	33	5214	34	26	41
	1	5	100	500.468	432.352	142	1.24025	0.617413	12.1551	0.914063	0.920208	3922	33	5214	34	26	41

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1	1	171.584	470.016	750	1.71834	0.813219	17.8412	0.919168	0.657895	5098	20.392	21	16	25
2	2	174.47	174.326	115	1.36792	0.60239	12.1005	0.691473	0.65497	4161	36.1824	38	28	35
3	3	177.392	331.348	133	1.70493	0.810918	13.9573	0.910714	0.708333	4100	28.7582	29	27	44
4	4	179.828	341.081	98	1.51308	0.750701	11.2772	0.805644	0.652308	4268	47.1148	49	37	59
5	5	182.452	374.305	196	1.64008	0.804616	15.7973	0.805644	0.640523	9241	31.1017	24	18	28
6	6	185.281	415.42	171	1.60552	0.844191	12.2573	0.914728	0.670551	4074	22.4195	24	17	28
7	7	188.341	455.42	118	1.64552	0.844191	12.2573	0.923423	0.716783	4596	37.5327	38	28	41
8	8	191.455	495.42	205	2.43532	0.913525	16.1558	0.89916	0.629412	4016	46.7293	47	37	55
9	9	194.627	535.42	107	2.61388	0.923925	11.672	0.918782	0.709004	8458	15.0994	47	37	55
10	10	197.859	575.42	181	1.38148	0.689916	15.1808	0.91299	0.760504	8163	14.7715	45	35	53
11	11	201.144	615.42	105	1.63706	0.781746	14.6415	0.910524	0.720133	7660	14.7715	45	35	53
12	12	204.488	655.42	105	1.57163	0.717469	11.5674	0.9375	0.720133	4529	14.7715	45	35	53
13	13	207.891	695.42	159	1.2167	0.619031	11.2283	0.913793	0.722727	4533	28.0063	29	21	35
14	14	211.356	735.42	97	1.30584	0.619031	11.132	0.92381	0.746154	4091	42.1733	45	35	50
15	15	214.881	775.42	71	1.30538	0.619031	11.132	0.879518	0.65636	2333	31.9589	32	26	38
16	16	218.469	815.42	105	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
17	17	222.122	855.42	81	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
18	18	225.841	895.42	93	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
19	19	229.614	935.42	116	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
20	20	233.441	975.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
21	21	237.322	1015.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
22	22	241.259	1055.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
23	23	245.252	1095.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
24	24	249.301	1135.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
25	25	253.406	1175.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
26	26	257.568	1215.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
27	27	261.787	1255.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
28	28	266.066	1295.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
29	29	270.406	1335.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
30	30	274.806	1375.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
31	31	279.266	1415.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
32	32	283.686	1455.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
33	33	288.166	1495.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
34	34	292.706	1535.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
35	35	297.306	1575.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
36	36	301.866	1615.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
37	37	306.486	1655.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
38	38	311.166	1695.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
39	39	315.906	1735.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
40	40	320.706	1775.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
41	41	325.566	1815.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
42	42	330.486	1855.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
43	43	335.466	1895.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
44	44	340.506	1935.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
45	45	345.606	1975.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
46	46	350.766	2015.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
47	47	355.986	2055.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
48	48	361.266	2095.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
49	49	366.606	2135.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
50	50	372.006	2175.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
51	51	377.466	2215.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
52	52	382.986	2255.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
53	53	388.566	2295.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
54	54	394.206	2335.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
55	55	399.906	2375.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
56	56	405.666	2415.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
57	57	411.486	2455.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
58	58	417.366	2495.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
59	59	423.306	2535.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
60	60	429.306	2575.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
61	61	435.366	2615.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
62	62	441.486	2655.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
63	63	447.666	2695.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
64	64	453.906	2735.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
65	65	460.206	2775.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
66	66	466.566	2815.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
67	67	473.006	2855.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
68	68	479.506	2895.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
69	69	486.066	2935.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
70	70	492.686	2975.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
71	71	499.366	3015.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
72	72	506.106	3055.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
73	73	512.906	3095.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
74	74	519.766	3135.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
75	75	526.686	3175.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
76	76	533.666	3215.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
77	77	540.706	3255.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
78	78	547.806	3295.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
79	79	554.966	3335.42	125	1.30538	0.619031	11.132	0.879518	0.65636	4096	39.0095	40	30	48
80	80	562.186	3375.42	125	1.30538	0.619031	11.132	0.87951						

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106	359.018	312.36	116	1.78064	0.872412	12.0478	0.593638	0.473374	41.81	38	28	43
107	361.716	169.031	129	1.60135	0.781018	12.8159	0.508451	0.461538	75.38	46	46	71
108	370.42	126.786	112	1.12766	0.42473	11.9116	0.303224	0.217948	42.18	30	30	64
109	379.666	101.258	91	1.21577	0.568727	11.1132	0.350224	0.217948	41.18	34	34	57
110	382.727	601.684	187	1.71888	0.820259	15.4304	0.921295	0.611111	18.30	48	48	51
111	388.96	193.188	303	1.87034	0.835633	19.6116	0.907188	0.611111	12.10	36	36	61
112	397.155	337.23	174	1.72598	0.821595	14.8613	0.915789	0.61125	8.51	30	30	59
113	384.648	261.441	136	1.49281	0.804233	13.1559	0.894737	0.653646	45.77	32	32	61
114	401.428	302.432	114	1.51997	0.821845	22.9591	0.866109	0.613333	19.64	47	47	61
115	395.517	232.514	149	2.01919	0.871845	22.1116	0.923666	0.613333	5.11	36	36	29
116	416.597	773.038	384	1.50902	0.780201	24.2789	0.940828	0.60303	45.92	28	28	32
117	424.436	70.7462	159	1.4314	0.780201	24.2789	0.940828	0.60303	45.92	22	22	35
118	429	16	109	1.24326	0.581169	11.7806	0.921071	0.713238	39.03	33	33	38
119	431.508	178.295	132	1.28331	0.608466	12.9641	0.921071	0.713238	41.52	31	31	31
120	447.446	371.2	393	1.84933	0.809466	12.9641	0.921071	0.713238	102.17	41	41	33
121	435.448	108.209	91	1.44731	0.753453	10.7611	0.911807	0.601213	37.61	26	26	31
122	431.589	245.441	131	1.47057	0.811488	12.9149	0.891136	0.782778	42.81	33	33	33
123	441.345	472.391	110	1.28412	0.725407	11.8945	0.92437	0.785714	42.49	34	34	35
124	447.904	87.9959	178	1.31618	0.811488	12.9149	0.92437	0.785714	42.49	25	25	39
125	455.604	60.2013	164	1.42045	0.811488	12.9149	0.92437	0.785714	42.49	27	27	40
126	456.785	488.54	113	1.31601	0.811488	12.9149	0.92437	0.785714	42.49	30	30	48
127	456.785	488.54	113	1.31601	0.811488	12.9149	0.92437	0.785714	42.49	38	38	44
128	461.271	159.5	100	1.27479	0.80244	11.2478	0.92437	0.785714	42.49	31	31	47
129	463.58	148.499	287	1.78781	0.80244	11.2478	0.92437	0.785714	42.49	24	24	38
130	472.782	797.252	101	1.28412	0.811488	12.9149	0.92437	0.785714	42.49	28	28	46
131	477.807	328.166	114	1.28412	0.811488	12.9149	0.92437	0.785714	42.49	60	60	75
132	478.149	22.1551	109	1.42045	0.811488	12.9149	0.92437	0.785714	42.49	38	38	44
133	485.604	60.2013	164	1.42045	0.811488	12.9149	0.92437	0.785714	42.49	30	30	48
134	490.397	169.904	61	1.41513	0.807364	8.95223	0.851351	0.63	61.61	35	35	134
135	498.38	254.707	174	1.53716	0.766778	14.8143	0.933444	0.74259	44.42	40	40	47
136	497.06	712.497	42	1.60841	0.766778	14.8143	0.933444	0.74259	44.42	31	31	41
137	496.229	331.5	208	2.09018	0.87165	14.2127	0.921071	0.713238	41.52	24	24	38
138	498.719	358.104	95	1.10594	0.87165	14.2127	0.921071	0.713238	41.52	29	29	46
139	504.117	358.104	115	1.19899	0.951714	12.1005	0.90566	0.713238	40.30	32	32	53
140	507.001	502.192	91	1.30553	0.855464	10.4817	0.920792	0.713238	42.50	33	33	50
141	517.173	52.4425	98	1.27254	0.713616	11.2722	0.915892	0.703143	39.40	38	38	48
142	527.365	62.4388	98	1.39204	0.655662	11.1704	0.890909	0.7	39.40	40	40	48
143	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	42	42	47
144	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	34	34	53
145	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	43	43	53
146	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	34	34	53
147	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	43	43	53
148	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	34	34	53
149	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	43	43	53
150	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	34	34	53
151	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	43	43	53
152	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	34	34	53
153	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	43	43	53
154	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	34	34	53
155	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	43	43	53
156	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	34	34	53
157	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	43	43	53
158	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	34	34	53
159	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	43	43	53
160	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	34	34	53
161	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	43	43	53
162	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	34	34	53
163	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	43	43	53
164	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	34	34	53
165	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	43	43	53
166	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	34	34	53
167	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	43	43	53
168	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	34	34	53
169	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	43	43	53
170	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	34	34	53
171	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	43	43	53
172	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	34	34	53
173	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	43	43	53
174	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	34	34	53
175	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	43	43	53
176	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	34	34	53
177	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	43	43	53
178	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	34	34	53
179	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	43	43	53
180	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	34	34	53
181	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	43	43	53
182	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	34	34	53
183	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	43	43	53
184	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	34	34	53
185	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	43	43	53
186	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	34	34	53
187	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	43	43	53
188	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	34	34	53
189	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	43	43	53
190	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	34	34	53
191	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	43	43	53
192	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	34	34	53
193	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	43	43	53
194	541.318	315.156	261	1.19415	0.54657	11.0558	0.821077	0.717273	41.29	34		

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41	132.431	416.005	118	1.37941	0.71601	12.2573	0.929134	0.7375	1370	37.0339	39	29	44
42	141.089	478.484	192	1.46597	0.84491	15.4523	0.91466	0.821631	4755	24.7656	25	19	31
43	146.375	477.265	298	1.51175	0.83275	15.4523	0.927204	0.825573	9510	31.9128	33	25	39
44	145.352	318.468	161	1.1547	0.597474	12.2573	0.91584	0.722077	1314	30.5957	32	24	37
45	149.67	36.7411	203	1.35381	0.670083	18.0188	0.841884	0.763138	9182	45.2315	47	35	55
46	151.921	219.534	169	1.69944	0.80052	15.4523	0.915644	0.75	4903	25.9418	26	20	32
47	150.379	93.6746	169	1.51964	0.851122	14.4664	0.921991	0.870635	4649	27.5089	28	21	34
48	155.872	139.7168	135	2.04842	0.871425	13.4204	0.921192	0.816034	7571	40.4866	42	31	49
49	155.578	139.7168	135	1.54998	0.76404	13.1106	0.913162	0.767045	4282	31.7976	33	25	39
50	164.123	388.369	198	2.64016	0.911683	15.8777	0.92093	0.8875	5235	26.1394	27	20	32
51	177.003	501.03	336	3.04298	0.94461	20.6035	0.929521	0.918462	9818	29.2798	31	22	37
52	175.35	160.975	160	1.74376	0.815223	14.273	0.91954	0.83761	4716	27.915	29	22	34
53	182.163	312.893	177	2.04491	0.874919	15.0121	0.907692	0.874831	8036	34.4407	36	24	34
54	183.062	31.9402	193	1.35599	0.674432	15.4523	0.881278	0.709559	8459	43.829	44	34	53
55	183.062	145.914	113	1.62117	0.80117	13.4935	0.899371	0.847059	4328	30.2851	31	24	34
56	183.435	436.351	131	1.32043	0.653061	12.9149	0.935714	0.79762	1313	31.1577	32	26	39
57	191.742	254.049	308	1.6923	0.804132	22.2265	0.861143	0.66667	1935	49.3923	52	39	60
58	187.164	466.48	152	1.6157	0.791121	13.9116	0.921212	0.745058	4421	25.0852	30	22	36
59	191.374	354.361	208	2.11997	0.881157	16.2737	0.920351	0.830303	8950	43.0288	44	33	52
60	198.325	391.686	226	2.09237	0.8781	13.4809	0.924528	0.8125	4356	28.9524	30	23	34
61	198.325	391.686	226	1.6339	0.741034	16.9633	0.933884	0.719562	8913	38.4381	42	30	44
62	202.194	432.545	156	1.8066	0.832832	12.4172	0.882122	0.617059	4294	35.3207	37	27	40
63	223.218	432.545	156	1.92249	0.754187	14.0935	0.905977	0.686428	4285	27.3297	28	21	33
64	223.218	75.8455	110	1.6758	0.601587	11.8345	0.92137	0.733333	8320	57.4545	61	46	77
65	228.28	212.437	206	1.7235	0.611138	16.1953	0.923767	0.754579	4394	21.3301	22	16	23
66	230.35	41.7761	117	2.0402	0.868124	14.273	0.930233	0.8	7553	47.2042	50	37	63
67	230.35	41.7761	117	1.7922	0.829231	12.2053	0.9	0.64773	5538	55.8803	59	44	59
68	232.214	425.811	113	1.50432	0.747143	13.4935	0.916667	0.809532	4894	31.3566	32	23	34
69	232.214	425.811	113	1.19809	0.550163	12.7162	0.92029	0.755932	4349	34.2141	35	28	42
70	233.37	348.239	160	1.281	0.656032	15.1868	0.922077	0.703125	9241	51.3389	52	40	64
71	235.367	256.523	243	1.81026	0.83355	17.5897	0.91186	0.778116	5636	23.1934	24	18	28
72	246.231	338.726	317	1.66176	0.798419	12.8655	0.921586	0.666667	4416	34.2	35	26	43
73	254.928	370.701	321	1.50066	0.745518	12.7162	0.913659	0.647959	629	35.4488	37	29	43
74	254.928	370.701	321	2.16747	0.847234	13.5675	0.895062	0.835965	4313	29.7148	30	24	31
75	260.117	22.453	115	2.16747	0.847234	13.5675	0.913659	0.647959	629	25.553	26	20	32
76	272.467	22.453	115	2.07428	0.874634	19.0492	0.92233	0.832333	7294	34.8618	37	28	41
77	271.121	82.2348	129	1.19777	0.774724	13.7736	0.91411	0.673208	4396	30.4861	32	23	36
78	278.414	427.494	114	1.37108	0.70881	12.5143	0.920291	0.727811	4288	31.5965	35	26	41
79	281.035	107.494	114	1.36106	0.60015	13.5406	0.91176	0.8	4390	30.4861	32	23	36
80	281.035	107.494	114	1.37108	0.60015	13.5406	0.922642	0.75	8558	47.5111	49	37	56
81	283.019	378.134	208	1.55493	0.764693	16.2737	0.924441	0.712857	9150	45.4327	48	33	56
82	291.019	302.297	208	1.72398	0.851114	13.1106	0.914056	0.833333	4338	31.3926	32	25	38
83	291.019	302.297	208	1.19433	0.538012	16.4503	0.904077	0.689016	5496	33.5122	35	25	42
84	302.081	432.484	115	2.71841	0.923981	20.1223	0.951169	0.718398	20112	28.3287	29	21	33
85	328.196	400.376	180	1.60316	0.833199	15.1868	0.950931	0.825	8018	44.5444	46	34	53
86	318.024	319.5	122	1.15159	0.647196	12.4634	0.903704	0.72619	4028	33.0166	35	27	34
87	319.139	480.345	155	1.73598	0.817119	16.4913	0.901639	0.631162	4708	28.5212	30	23	34
88	327.788	357.364	165	2.07227	0.868976	15.5915	0.910256	0.739593	6011	45.5183	48	37	51
89	338.114	197.118	76	1.53207	0.884113	14.3175	0.909605	0.557093	5244	32.5714	34	26	35
90	344.676	379.211	111	1.67896	0.803716	9.50789	0.910256	0.739593	6011	31.0263	33	40	62
91	353.418	284.282	123	2.08045	0.874694	16.4602	0.918103	0.516691	8798	43.1084	45	32	53
92	362.172	230.46	61	1.94982	0.779153	14.1935	0.913295	0.631866	4870	36.0104	37	27	41
93	362.172	230.46	61	1.01982	0.304104	10.5248	0.915785	0.719008	3703	30.6056	32	24	36
94	364.328	358.458	129	1.41465	0.707325	8.01292	0.840539	0.7129	3820	42.632	44	33	52
95	377.571	322.971	259	1.66998	0.80082	18.1595	0.843333	0.6475	13664	42.632	44	33	52
96	380.453	225.841	121	2.67929	0.927743	17.1499	0.916667	0.712953	5820	52.7569	51	39	67
97	382.282	255.841	121	1.30452	0.62149	12.4172	0.916667	0.712953	5820	25.1848	26	19	21
98	384.114	197.118	76	2.10452	0.842169	12.4172	0.916667	0.712953	5820	33.7851	34	27	41
99	384.114	197.118	76	1.46088	0.884113	14.3175	0.909605	0.557093	5244	32.5714	34	26	35
100	400.468	231.315	203	1.53207	0.795194	9.50789	0.904162	0.710763	2078	31.0263	33	40	62
101	405.127	381.342	142	1.43378	0.795194	15.0769	0.923272	0.667163	8751	43.1084	45	32	53
102	405.127	381.342	142	1.55043	0.841399	15.6759	0.923443	0.620719	6950	36.0104	37	27	41
103	416.389	340.643	126	1.31237	0.642552	13.4462	0.922078	0.728203	4346	30.6056	32	24	36
104	423.782	433.085	136	1.6334	0.838154	23.5612	0.872	0.619318	18633	31.0556	34	26	40
105	429.267	50.4533	130	1.78378	0.87084	13.8198	0.91677	0.71825	4218	28.12	28	22	34
106	432.237	209.237	38	1.71216	0.811559	6.9558	0.86087	0.59375	3012	79.2632	80	61	100
107	440.931	222.448	174	1.75735	0.82271	14.8843	0.875	0.729167	2932	82.0571	82	60	100
108	443.61	277.99	105	1.46088	0.828455	11.4651	0.87416	0.625	3901	37.1524	38	30	46
109	443.61	277.99	105	1.78618	0.828455	11.4651	0.87416	0.625	3901	37.1524	38	30	46
110	466.516	321.648	122	1.41764	0.703313	12.4651	0.91733	0.72519	4515	31.592	35	26	40
111	473.447	121.928	119	1.70559	0.810046	12.3032	0.84612	0.610256	3984	31.479	35	26	40
112	482.816	335.798	114	1.13164	0.648355	12.0478	0.81432	0.572233	3853	37.0377	39	29	44
113	486.5	287.192	104	1.15364	0.560255	11.3072	0.81432	0.572233	3853	37.0377	39	29	44
114	497.774	121.27	113	1.46338	0.721103	12.4002	0.81432	0.572233	3853	31.037	35	27	41
115	498.638	334.246	127	1.47025	0.720667	12.7162	0.81432	0.572233	3853	31.037	35	27	41
116	502.025	136.736	156	1.15083	0.505312	14.0935	0.83359	0.8	4744	30.4103	32	23	37

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1	11.1154	387.587	108	1.42566	0.712937	11.5073	0.904348	0.533235	3955	38.0288	29	31	46
2	9.80103	425.712	66	1.20659	0.559891	9.187	0.88	0.733232	3058	46.3155	48	40	53
3	11.4346	434.818	77	1.37652	0.528466	9.90149	0.927711	0.7	3950	38.2312	40	34	46
4	14.4143	456.346	70	1.03328	0.231744	9.4407	0.886076	0.7	3970	36.1113	58	46	58
5	18.3947	455.1316	114	1.263	0.610824	12.0478	0.912	0.730769	3892	31.1404	35	27	41
6	21.18	472.59	100	1.52012	0.753156	11.2838	0.884956	0.714286	3840	38.4	39	22	36
7	23.1143	499.143	169	1.52552	0.755554	13.3512	0.903216	0.673077	4072	23.0837	30	22	36
8	25.3148	432.905	169	1.95543	0.855145	15.5126	0.936283	0.63	12608	66.7059	69	81	94
9	27.4887	450.614	114	1.27031	0.616685	12.0478	0.913057	0.730769	1004	35.1278	37	28	41
10	27.4887	415.544	125	1.40131	0.721089	12.6157	0.905797	0.694444	7138	57.104	60	44	49
11	27.4887	415.544	125	1.23156	0.56442	13.0131	0.917243	0.738849	4708	31.6391	32	25	39
12	28.4593	444.607	113	1.60045	0.60045	11.9848	0.91129	0.724759	4075	36.0619	37	29	41
13	30.7057	490.193	196	1.81803	0.811803	15.7973	0.903226	0.606811	5557	28.352	30	22	35
14	32.4816	311.425	196	1.07459	0.374947	12.4935	0.923541	0.785714	4063	28.4126	29	23	34
15	34.9115	311.425	222	1.28906	0.61066	17.9125	0.87427	0.577271	11427	45.3452	45	38	58
16	36.7822	323.473	132	1.10508	0.45606	12.4534	0.917293	0.782051	1642	38.2131	39	30	46
17	36.7822	323.473	132	1.60128	0.78187	13.0419	0.937063	0.681673	4152	30.9951	32	25	37
18	36.7822	323.473	132	1.49416	0.714624	16.4294	0.917719	0.69281	8404	41.3283	42	32	52
19	37.3013	464.719	92	1.43718	0.722855	10.822	0.910881	0.69697	3641	39.5761	39	31	49
20	37.3013	464.719	92	1.03578	0.240563	24.67	0.910416	0.735385	14880	31.7297	32	24	38
21	38.4333	376.181	121	1.21491	0.567192	12.3408	0.895552	0.710058	4070	33.8167	35	27	41
22	38.4333	376.181	121	1.5995	0.780155	16.7746	0.934441	0.734667	7891	35.7059	37	28	43
23	38.4333	376.181	121	1.54008	0.743771	14.0935	0.922077	0.718789	4298	27.5513	28	21	34
24	38.4333	376.181	121	2.23108	0.93326	13.5408	0.817368	0.8	5206	38.1528	37	28	46
25	39.5089	208.8	91	1.33153	0.621074	10.8117	0.902513	0.659035	3739	40.2043	41	33	49
26	39.5089	208.8	91	1.42001	0.720491	12.2053	0.905917	0.709091	3961	33.8547	31	26	41
27	40.4333	376.181	121	2.01167	0.87453	15.5126	0.917416	0.678257	5541	28.3175	30	23	36
28	40.4333	376.181	121	1.70184	0.64651	13.0619	0.911565	0.697917	4152	30.8851	33	24	37
29	40.4333	376.181	121	2.23647	0.90113	11.672	0.90678	0.716252	4231	38.5234	29	30	43
30	40.4333	376.181	121	1.72753	0.60841	13.6809	0.90184	0.660192	4148	28.7823	29	23	36
31	40.4333	376.181	121	1.15651	0.513778	11.9948	0.90184	0.724359	4148	38.485	38	28	46
32	40.4333	376.181	121	1.6469	0.743048	22.6321	0.846619	0.649553	14872	38.9032	37	28	46
33	40.4333	376.181	121	1.71451	0.727416	11.7808	0.923443	0.61081	4053	31.1835	39	30	45
34	40.4333	376.181	121	1.51649	0.623109	18.6339	0.877816	0.598844	13955	51.1172	52	38	61
35	40.4333	376.181	121	1.62783	0.728135	15.5115	0.937304	0.684211	9270	32.0067	33	25	39
36	40.4333	376.181	121	2.56801	0.87225	18.7817	0.882166	0.507232	7730	27.9061	29	21	34
37	40.4333	376.181	121	1.67056	0.621152	16.4817	0.885714	0.65035	3656	41.4624	42	32	50
38	40.4333	376.181	121	2.16482	0.787276	16.3559	0.911111	0.711806	4628	22.5756	23	17	28
39	40.4333	376.181	121	1.61725	0.627216	15.3198	0.915277	0.757576	7793	51.9533	53	40	65
40	40.4333	376.181	121	1.52467	0.654417	13.2053	0.906971	0.764708	4458	38.1026	39	31	45
41	40.4333	376.181	121	1.52467	0.654417	13.2053	0.91411	0.716346	4233	28.4094	30	21	34
42	40.4333	376.181	121	1.71794	0.65137	11.4714	0.919425	0.815345	3923	37.0094	37	29	45
43	40.4333	376.181	121	1.13403	0.50173	10.3418	0.903216	0.694215	2493	32.0595	33	25	38
44	40.4333	376.181	121	1.89557	0.74596	17.9725	0.85237	0.67217	9197	37.25	38	28	46
45	40.4333	376.181	121	2.04372	0.774593	18.7053	0.918655	0.680404	8240	27.1475	28	21	34
46	40.4333	376.181	121	1.50812	0.64352	20.1535	0.872749	0.693748	11477	35.9781	36	28	43
47	40.4333	376.181	121	1.23712	0.643111	12.3072	0.915395	0.727227	2584	30.1176	31	24	36
48	40.4333	376.181	121	1.77549	0.82435	16.2592	0.915395	0.727227	2584	30.1176	31	24	36
49	40.4333	376.181	121	1.65246	0.791053	12.1805	0.92107	0.704093	8582	41.4589	44	32	50
50	40.4333	376.181	121	1.65246	0.791053	12.1805	0.92107	0.704093	8582	41.4589	44	32	50
51	40.4333	376.181	121	2.00944	0.841056	17.4419	0.927931	0.804273	4040	35.1304	37	28	42
52	40.4333	376.181	121	1.60678	0.641056	17.4419	0.927931	0.804273	4040	35.1304	37	28	42
53	40.4333	376.181	121	1.4086	0.60728	10.2671	0.924528	0.733816	8913	36.3796	37	28	42
54	40.4333	376.181	121	1.69143	0.604617	14.3271	0.91629	0.643382	4180	45.243	47	37	57
55	40.4333	376.181	121	1.34636	0.665575	21.831	0.91629	0.643382	4180	45.243	47	37	57
56	40.4333	376.181	121	1.52013	0.753161	14.3819	0.91629	0.643382	4180	45.243	47	37	57
57	40.4333	376.181	121	1.20724	0.560236	10.9981	0.896226	0.719397	3926	30.3097	36	27	41
58	40.4333	376.181	121	2.7007	0.928522	16.888	0.896226	0.719397	3926	30.3097	36	27	41
59	40.4333	376.181	121	1.22641	0.578918	11.2401	0.924606	0.763132	4011	39.7129	41	33	48
60	40.4333	376.181	121	1.23747	0.589013	12.7662	0.924606	0.763132	4011	39.7129	41	33	48
61	40.4333	376.181	121	1.64655	0.646013	11.4345	0.924606	0.763132	4011	39.7129	41	33	48
62	40.4333	376.181	121	1.64655	0.646013	11.4345	0.924606	0.763132	4011	39.7129	41	33	48
63	40.4333	376.181	121	2.21337	0.790527	16.8503	0.900928	0.724026	4156	37.7218	39	29	47
64	40.4333	376.181	121	1.71204	0.646013	12.2555	0.900928	0.724026	4156	37.7218	39	29	47
65	40.4333	376.181	121	1.71204	0.646013	12.2555	0.900928	0.724026	4156	37.7218	39	29	47
66	40.4333	376.181	121	1.93002	0.855302	16.9633	0.900928	0.724026	4156	37.7218	39	29	47
67	40.4333	376.181	121	1.26158	0.609669	12.2053	0.92126	0.75374	3975	33.9744	35	26	42
68	40.4333	376.181	121	1.26158	0.609669	12.2053	0.92126	0.75374	3975	33.9744	35	26	42
69	40.4333	376.181	121	1.45602	0.680437	15.0545	0.92228	0.706349	6679	37.5725	39	30	45
70	40.4333	376.181	121	1.24742	0.728174	12.7662	0.901108	0.711111	4017	31.3828	32	24	35
71	40.4333	376.181	121	1.13672	0.472599	24.2273	0.923313	0.770903	18482	40.0043	42	30	50
72	40.4333	376.181	121	1.13672	0.472599	24.2273	0.923313	0.770903	18482	40.0043	42	30	50
73	40.4333	376.181	121	1.13672	0.472599	24.2273	0.923313	0.770903	18482	40.0043	42	30	50
74	40.4333	376.181	121	1.13672	0.472599	24.2273	0.923313	0.770903	18482	40.0043	42	30	50
75	40.4333	376.181	121	1.13672	0.472599	24.2273	0.923313	0.770903	18482	40.0043	42	30	50
76	40.4333	376.181	121	1.13672	0.472599	24.2273	0.923313	0.770903	18482	40.0043	42	30	50
77	40.4333	376.181	121	1.13672	0.472599	24.2273	0.923313	0.770903	18482	40.0043	42	30	50
78	40.4333	376.181	121	1.13672	0.472599	24.2273	0.923313	0.770903	18482	40.0043	42	30	50
79	40.4333	376.181	121	1.13672	0.472599	24.2273	0.923313	0.770903	18482	40.0043	42	30	50
80	40.4333	376.181	121	1.13672	0.472599	24.2273	0.923313	0.770903	18482	40.0043	42	30	50
81	40.4333	376.181	121	1.13672	0.472599	24.2273	0.923313	0.770903	18482	40.0043	42	30	50
82	40.4333	376.181	121	1.13672	0.472599	24.2273	0.923313	0.770903	18482	40.0043	42	30	50
83	40.4333	376.181	121	1.13672	0.472599	24.2273	0.923313	0.770903	18482				

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1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60																																																																																																																																												

93	283.616	101.011	127	1.6816	0.805747	12.7162	0.313649	0.661458	4625	36.4173	37	29	44
94	284.653	410.671	173	1.53156	0.755964	14.8415	0.323134	0.750772	7670	41.3353	46	35	54
95	285.691	829.597	84	1.53556	0.758131	10.3416	0.303236	0.646134	4211	50.131	51	39	62
96	286.312	80.4401	154	1.13377	0.706446	14.0028	0.316647	0.733333	4536	39.896	30	23	36
97	287.939	305.311	90	1.08786	0.593716	10.7047	0.309081	0.742602	4067	45.3131	46	36	56
98	288.316	47.9316	107	1.77771	0.485053	11.4972	0.389316	0.84127	4700	43.356	44	38	61
99	289.325	312.755	106	1.53546	0.758839	11.4774	0.309053	0.84127	4700	40.4327	40	31	51
100	290.361	388.784	148	2.38785	0.908053	13.7273	0.480509	0.928571	5947	40.4327	40	31	51
101	291.355	344.741	44	1.29533	0.637205	16.4812	0.461534	0.68975	3740	45.4345	48	45	70
102	292.353	388.246	224	2.03565	0.874034	16.4812	0.314286	0.59893	9246	41.7768	41	32	50
103	293.312	242.253	359	2.13587	0.883628	13.7197	0.481034	0.763841	19817	55.2006	56	42	69
104	294.315	332.148	182	1.68413	0.731934	15.2327	0.328571	0.736742	9476	52.0269	55	41	64
105	295.322	65	141	1.02189	0.615902	14.4062	0.310615	0.639216	4361	26.7773	28	20	32
106	296.327	55	159	1.24078	0.624842	14.1035	0.323977	0.752381	4584	31.5696	33	25	38
107	297.313	743	242	1.64339	0.793338	13.5172	0.489381	0.743271	9916	41.1552	42	31	51
108	298.346	6.3144	151	1.33319	0.619137	13.8658	0.392032	0.725952	4248	28.1391	29	22	34
109	299.344	135.607	28	1.25680	0.460686	15.97082	0.4875	0.646657	5659	102.107	115	166	238
110	300.322	664	773	1.60766	0.736163	14.8415	0.323135	0.758772	4625	27.1618	28	21	33
111	301.315	396.531	145	1.70417	0.684894	13.5075	0.329487	0.74059	8423	54.0897	60	47	69
112	302.318	829.597	84	1.70417	0.684894	13.5075	0.329487	0.74059	8423	54.0897	60	47	69
113	303.321	80.4401	154	1.22377	0.693118	16.6221	0.30795	0.645873	4593	21.7351	21	17	26
114	304.324	312.755	106	1.22377	0.693118	16.6221	0.30795	0.645873	4593	21.7351	21	17	26
115	305.327	388.784	148	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
116	306.330	344.741	44	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
117	307.333	388.246	224	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
118	308.336	242.253	359	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
119	309.339	332.148	182	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
120	310.342	47.9316	107	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
121	311.345	80.4401	154	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
122	312.348	305.311	90	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
123	313.351	312.755	106	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
124	314.354	388.784	148	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
125	315.357	344.741	44	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
126	316.360	388.246	224	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
127	317.363	242.253	359	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
128	318.366	332.148	182	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
129	319.369	47.9316	107	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
130	320.372	80.4401	154	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
131	321.375	305.311	90	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
132	322.378	312.755	106	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
133	323.381	388.784	148	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
134	324.384	344.741	44	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
135	325.387	388.246	224	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
136	326.390	242.253	359	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
137	327.393	332.148	182	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
138	328.396	47.9316	107	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
139	329.399	80.4401	154	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
140	330.402	305.311	90	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
141	331.405	312.755	106	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
142	332.408	388.784	148	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
143	333.411	344.741	44	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
144	334.414	388.246	224	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
145	335.417	242.253	359	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
146	336.420	332.148	182	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
147	337.423	47.9316	107	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
148	338.426	80.4401	154	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
149	339.429	305.311	90	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
150	340.432	312.755	106	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
151	341.435	388.784	148	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
152	342.438	344.741	44	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
153	343.441	388.246	224	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
154	344.444	242.253	359	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
155	345.447	332.148	182	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
156	346.450	47.9316	107	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
157	347.453	80.4401	154	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
158	348.456	305.311	90	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
159	349.459	312.755	106	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
160	350.462	388.784	148	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
161	351.465	344.741	44	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
162	352.468	388.246	224	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
163	353.471	242.253	359	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
164	354.474	332.148	182	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
165	355.477	47.9316	107	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
166	356.480	80.4401	154	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
167	357.483	305.311	90	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
168	358.486	312.755	106	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
169	359.489	388.784	148	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
170	360.492	344.741	44	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
171	361.495	388.246	224	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
172	362.498	242.253	359	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
173	363.501	332.148	182	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
174	364.504	47.9316	107	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
175	365.507	80.4401	154	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
176	366.510	305.311	90	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
177	367.513	312.755	106	1.3376	0.734632	13.7773	0.387114	0.4875	210	19.0809	19	16	22
178	368.516												

EV Table 2.doc

Example of the summary output of AnalyseDNA.m program
(summary for 10 3 by 3 montage images)

1	1187	163.912	75.3518	1.51819	0.397735	0.724461	0.137996	14.0812	3.315	0.905327	0.0350585	0.701248	0.073176	6449.26	3196.95	11.539	10.352	42.9444	18.9293
2	1201	14.331	50.5906	22.6384	0.395942	0.723895	0.138142	14.2034	3.43288	0.901571	0.0341153	0.70177	0.0730289	6786.37	3195.1	12.2416	17.0965	43.8245	17.582
3	1205	169.014	94.8722	1.60311	0.397935	0.72552	0.13165	14.0651	3.33904	0.905247	0.0361822	0.702891	0.0720005	6881.08	3525.41	14.7167	20.5918	46.3838	21.2287
4	1209	164.57	80.0678	1.59728	0.399682	0.727142	0.142518	14.3321	3.61812	0.902766	0.0374254	0.695945	0.0753889	6997.48	4212.47	13.1798	15.0362	44.9361	20.3112
5	1213	172.015	88.7145	1.60189	0.400493	0.721044	0.141204	14.338	3.65065	0.904152	0.0378984	0.70021	0.0756884	7050.32	4165.04	11.0559	21.4761	45.8522	21.1202
6	1217	171.921	90.64	1.51887	0.425512	0.724414	0.137721	14.0774	3.33846	0.904116	0.0352731	0.696204	0.0782855	6843.2	3924.12	14.216	13.3396	45.9485	19.1324
7	1221	165.112	81.6106	1.60542	0.451022	0.694813	0.145647	11.728	5.27411	0.893311	0.0481729	0.704526	0.0892393	5162.51	4359.11	24.3743	21.1949	36.16	22.0024
8	1225	129.864	99.2039	1.51806	0.405467	0.723621	0.137599	14.3933	3.60593	0.906384	0.0357179	0.702574	0.0751502	5865.87	3767.48	12.2732	17.2223	43.3781	17.7621
9	1229	177.287	84.1754	1.59301	0.404986	0.72566	0.139548	14.1402	3.47784	0.905208	0.0358258	0.700331	0.0766773	6576.34	4028.38	11.6064	17.8994	43.1463	16.2499
10	1233	166.53	86.5094	1.60347	0.400772	0.717147	0.138085	13.8105	3.46556	0.904275	0.0378111	0.702759	0.075372	6587.18	3718.39	14.2141	20.7694	45.8765	21.136
	1237	159.606	91.618	1.57284															
	1241	163.319	93.7558	25.6094															

CLAIMS

What is claimed is:

1. A method of predicting a property of a manipulation of cells based
5 upon a descriptor, said method comprising:
 providing a plurality of cells;
 manipulating said plurality of cells;
 capturing a morphological value from said plurality of cells;
 assigning a degree of presence of said morphological value; and
10 storing said morphological value and said degree of presence;
 wherein said descriptor is derived from a first component of a cell and
a second component of said cell, said capturing said morphometric value from said
plurality of cells comprises determining a relationship between said first component
and said second component.
- 15 2. The method of claim 1 wherein said first component and said second
component are selected from a protein, a protein modification, a nucleic acid, a lipid,
a carbohydrate, a subcellular structure and an organelle.
3. The method of 1 wherein said step of manipulation occurs in a manner
20 selected from a electrical source, a chemical source, a thermal source, a gravitational
source, a nuclear source, a temporal source, and a biological source
4. The method of claim 3 wherein said chemical source is selected from a
pharmacological candidate and a drug screening library.
5. The method of claim 1 wherein said morphological value is selected
25 from a count, an area, a perimeter, a length, a breadth, a fiber length, a fiber breadth, a
shape factor, a elliptical form factor, an inner radius, an outer radius, a mean radius,
an equivalent radius, an equivalent sphere volume, an equivalent prolate volume, an
equivalent oblate volume, an equivalent sphere surface area, an average gray value, a
total gray value, and an optical density.
6. The method of claim 1 wherein said degree of presence is
30 multiple of a quantized value.

7. A computer program product for populating a database with manipulated biological information, said computer program product comprising:
- code for providing a plurality of cells in various stages of the cell cycle, said stages of the cell cycle including at least one selected from interphase, G0 phase, G1 phase, S phase, G2 phase, M phase, prophase, prometaphase, metaphase, anaphase, and telophase;
 - code for manipulating said cells in said various stages of cell cycle development to form a plurality of manipulated cells;
 - code for capturing an image of said plurality of manipulated cells;
 - code for determining a descriptor from said image for said manipulated cells;
 - code for populating a database with said descriptor;
 - wherein said image includes a first component of a cell and a second component of said cell; and
 - a computer readable storage medium for holding the codes.
8. The computer program product of claim 7 wherein said first component and said second component are selected from a protein, a protein modification, a nucleic acid, a lipid, a carbohydrate, a sub-cellular structure and an organelle.
9. The computer program product of claim 7 wherein said image is a digitized representation of said plurality of manipulated cells.
11. The computer program product of claim 9 wherein said digitized representation provides a density value of said plurality of manipulated cells.
11. The computer program product of claim 7 wherein said descriptors comprise numeric or logical values.
12. The computer program product of claim 11 wherein said values further comprises a nucleotide.
13. The computer program product of claim 11 wherein said values further comprises an amino acid letter.
14. A system for capturing images of cells or cell structures, the system comprising:
- a cell holder comprising a plurality of sites in a spatial orientation, each of the sites being capable of holding a plurality of cells to be imaged;

an image capturing device coupled to the cell holder, the image capture device being adapted to capture at least one image in at least one of the plurality of sites;

an illumination apparatus comprising a liquid light guide coupled to the plate for highlighting the plurality of cells in a relatively even spatial manner for image capturing purposes;

an image processing device coupled to the image capturing device, the image capturing device being adapted to convert the image into a digital representation; and

a database storage device comprising a database management element coupled to the image capturing device, the database storage device being adapted to retrieve the digital representation of the image from the image processing device and storing the digital representation.

15. The system of claim 14 further comprising a stage comprising a device for moving the cell holder in a spatial direction to traverse across the cell holder in the spatial orientation.

16. The system of claim 14 wherein the illumination apparatus comprises sub-elements, at least one of the sub-elements being positioned away from the image capturing device to prevent a possibility of vibration from the one sub-elements to be transmitted to the image capturing device.

17. The system of claim 14 wherein the digital representation comprises a plurality of regions and objects.

18. The system of claim 14 further comprising a computing device connected between the database storage device and the image processing device.

19. The system of claim 14 wherein the image capturing device comprises a magnification of at least 1X and greater to capture the image of the site.

20. The system of claim 14 wherein the plurality of sites comprises at least 96 sites.

21. The system of claim 14 wherein the liquid light guide characterized as a flexible member that substantially prevents vibration from the an element of the illumination apparatus to be transferred to the image capturing device.

22. The system of claim 14 wherein the spatial direction can be selected from an x-direction, a y-direction, or a z-direction in a Cartesian coordinate system.

23. The system of claim 14 wherein the each of the sites comprises
5 a volume that is sufficient to prevent a solution therein from evaporating in a substantial manner that may influence the image capturing.

24. A method for identifying a mechanism of action for a first compound, the method comprising the steps of:
receiving the first compound;
10 measuring at least one feature of a cellular phenotype to define a target phenotype;
identifying additional compounds providing a feature similar to the feature identified in the measuring step; and
characterizing the first compound in terms of distance from a specific
15 target phenotype having known characteristics.

25. The method of claim 24 comprising the further step of storing the additional compounds and their associated phenotypes in a database.

26. A method for identifying a mechanism of action for a cellular stimulus, the method comprising the steps of:
20 receiving cells of interest;
measuring at least one feature of the cells to define and quantify a target phenotype;
identifying additional compounds providing a feature similar to the feature identified in the measuring step; and
25 characterizing the first compound in terms of distance from a specific target phenotype having known characteristics.

27. A method for identifying information relevant to at least one of a mechanism of action and cellular activity by utilizing assay data to elucidate a phenotype, the method comprising the steps of:
30 identifying a target protein;
identifying positive and negative biochemical hits related to the target protein;
defining the target phenotype utilizing the positive and negative hits;

identifying other compounds providing similar features; and
characterizing the first compound in terms of distance from a specific
target phenotype having known characteristics.

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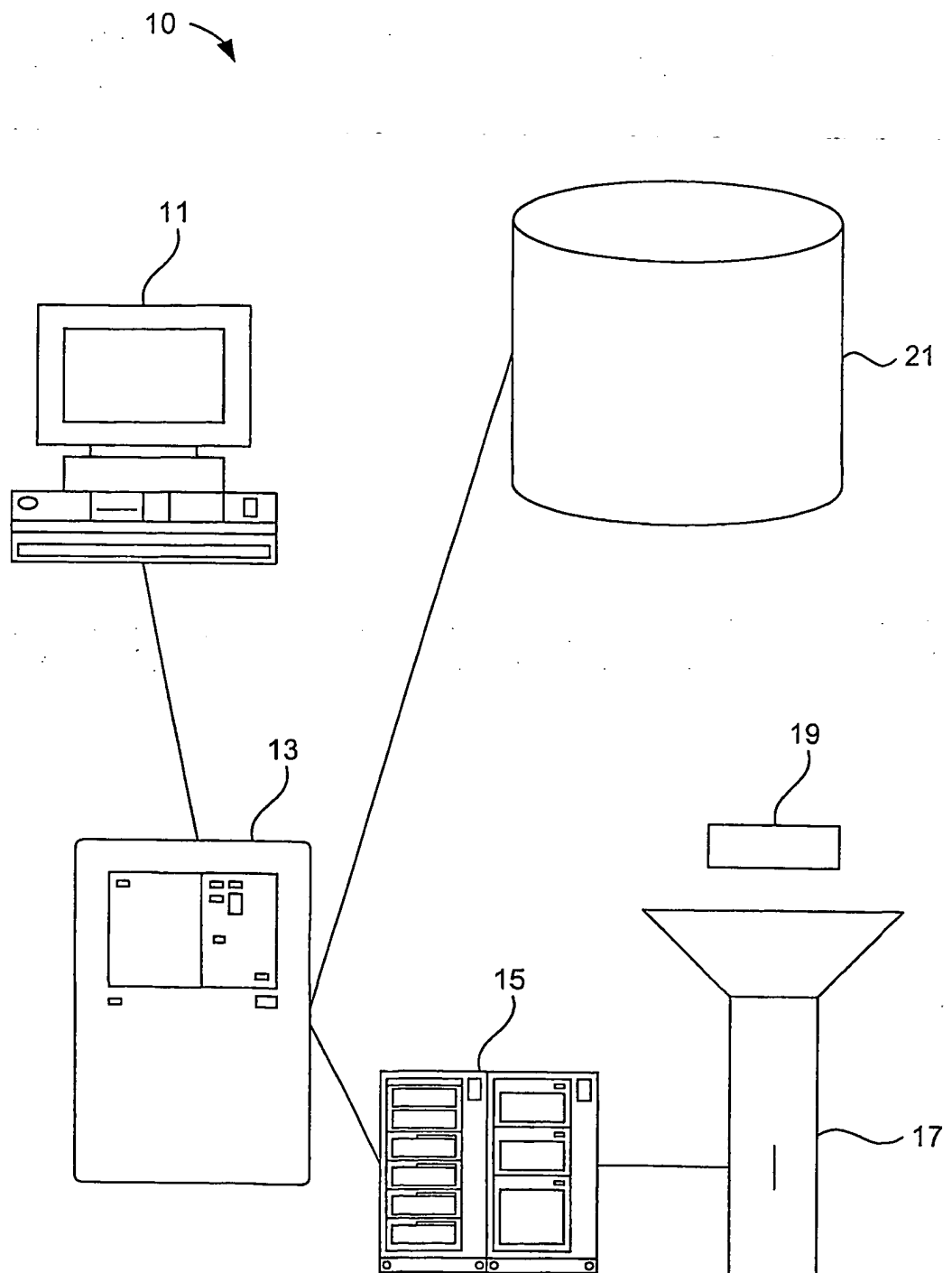


FIG. 1

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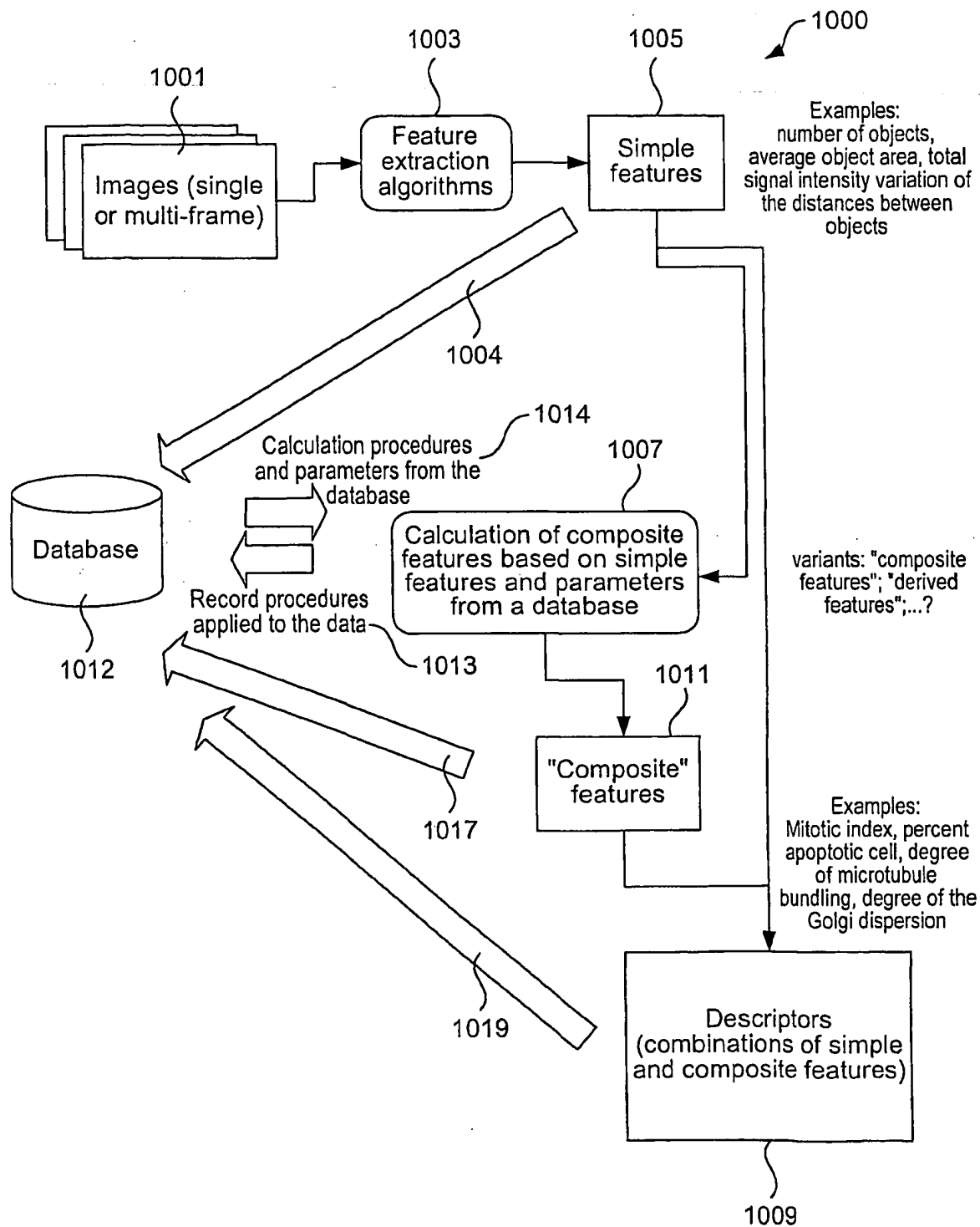


FIG. 1A

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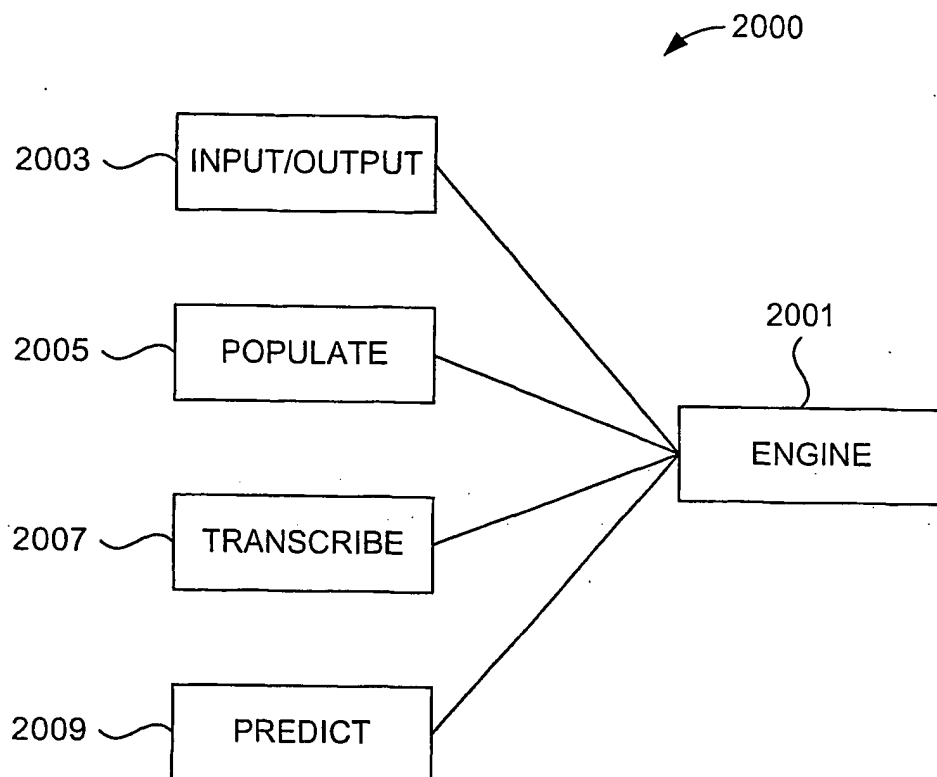


FIG. 1B

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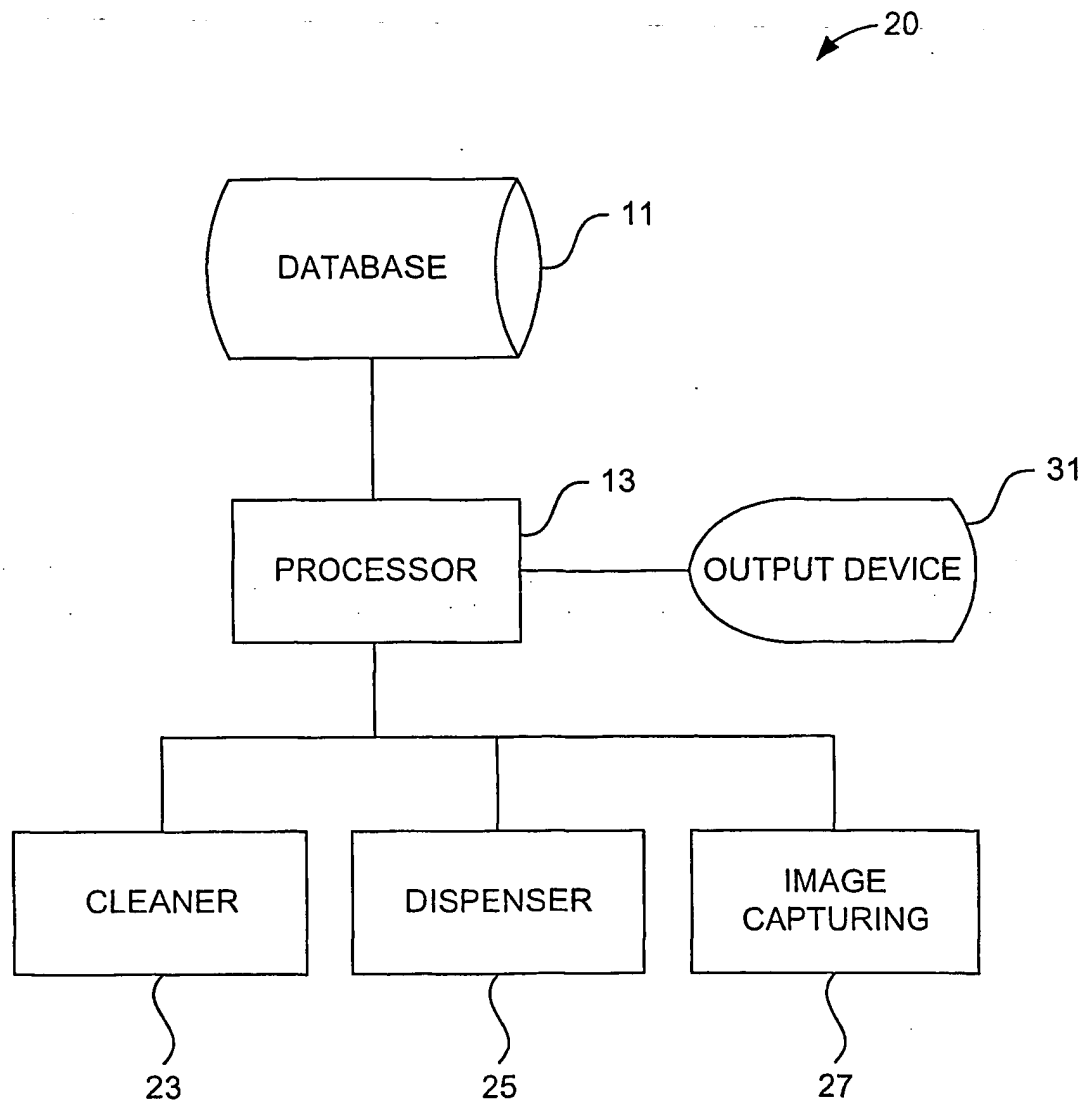


FIG. 2

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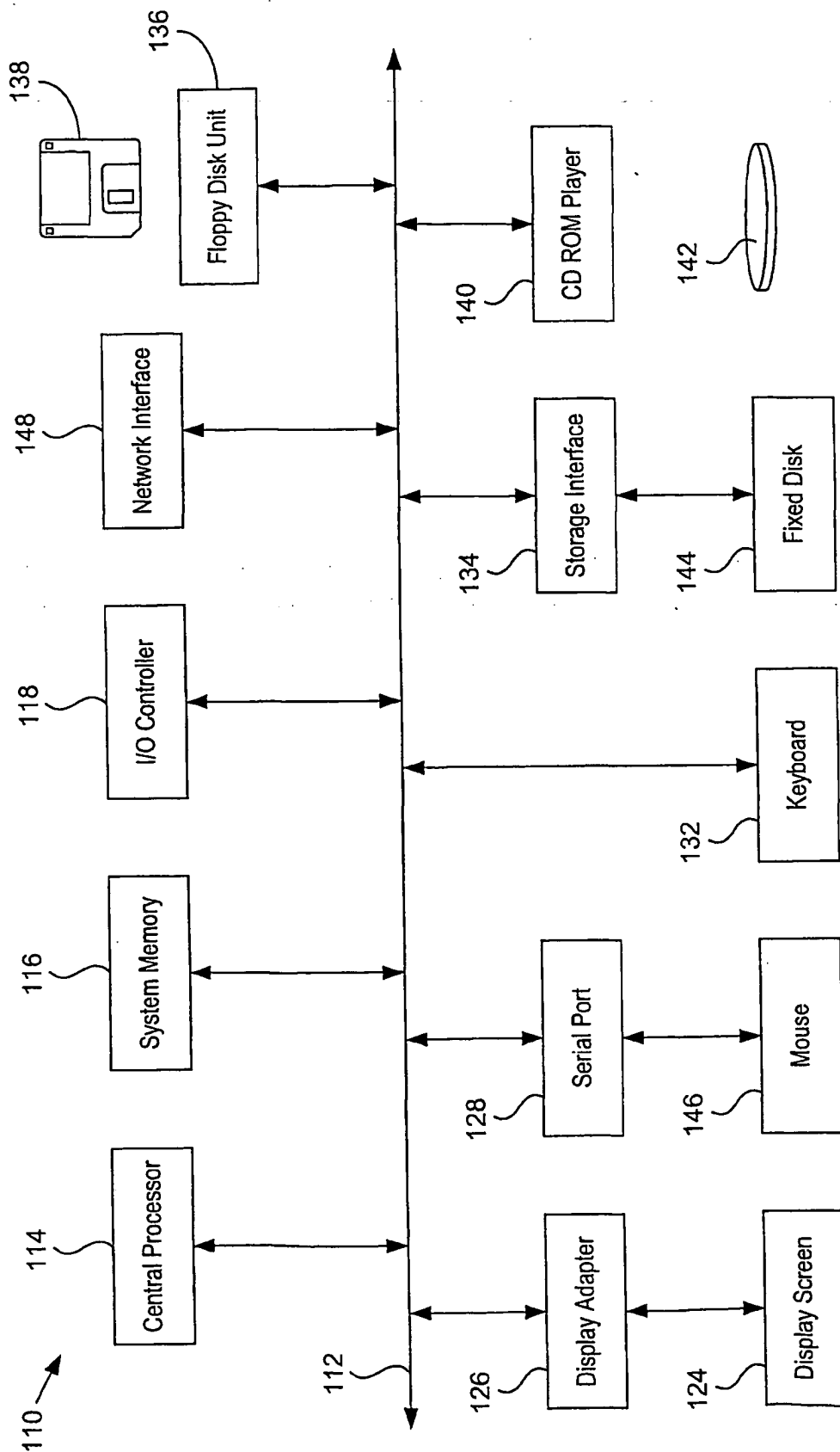


FIG. 3

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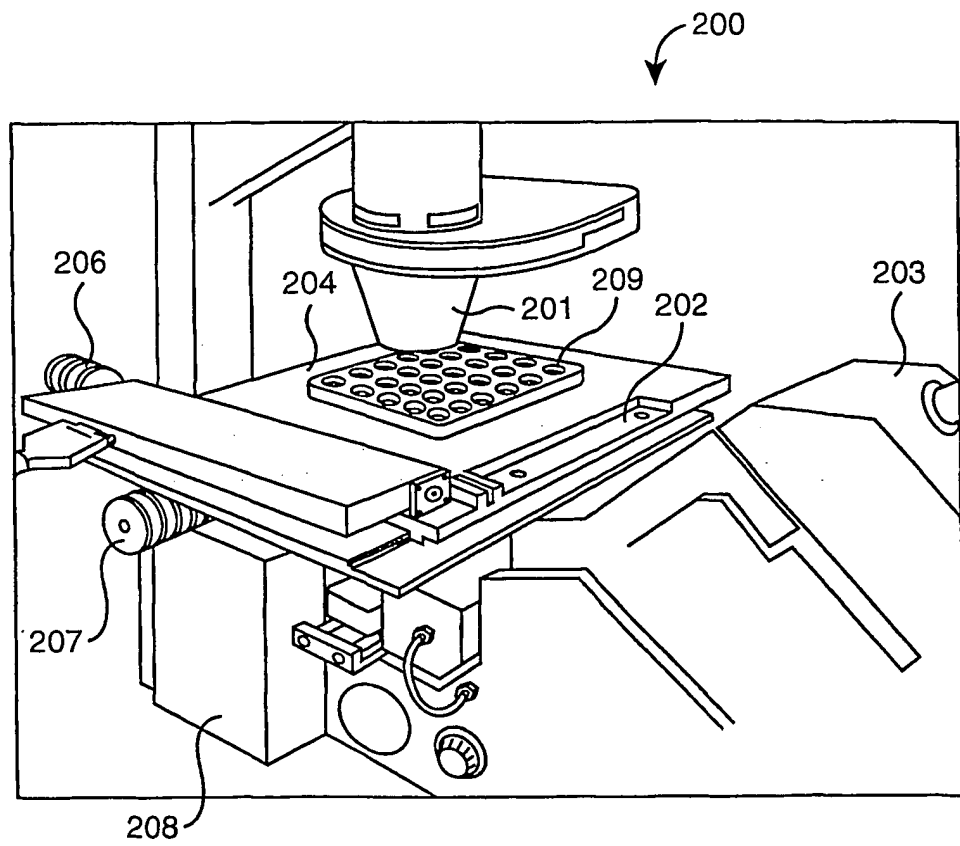


FIG. 4

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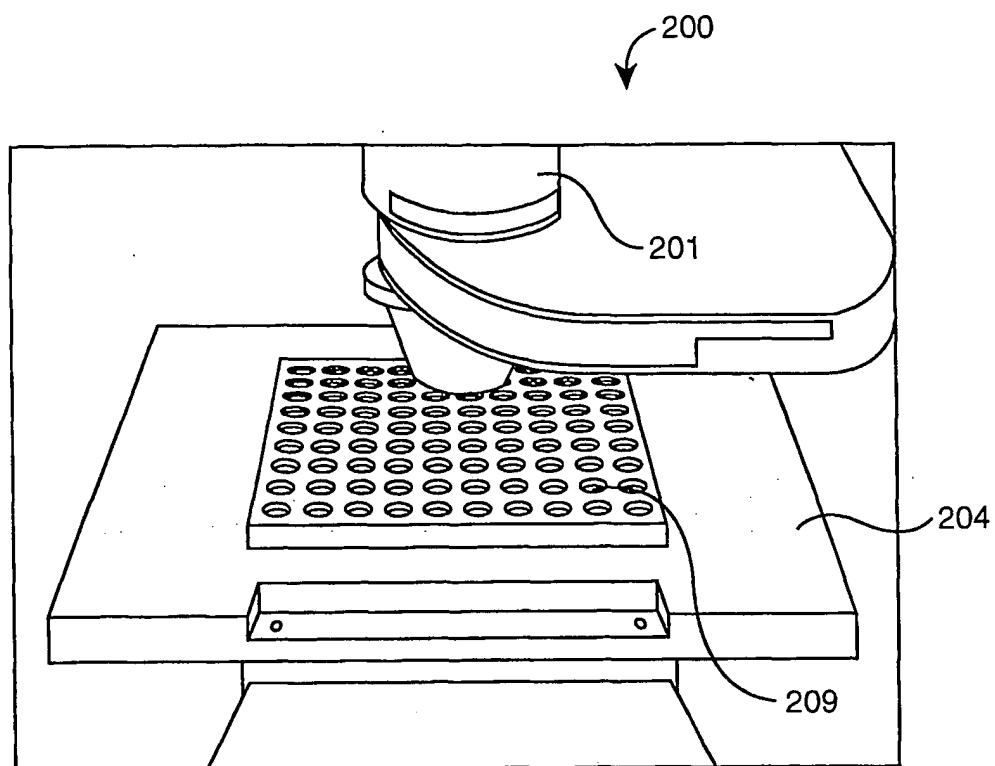


FIG. 5

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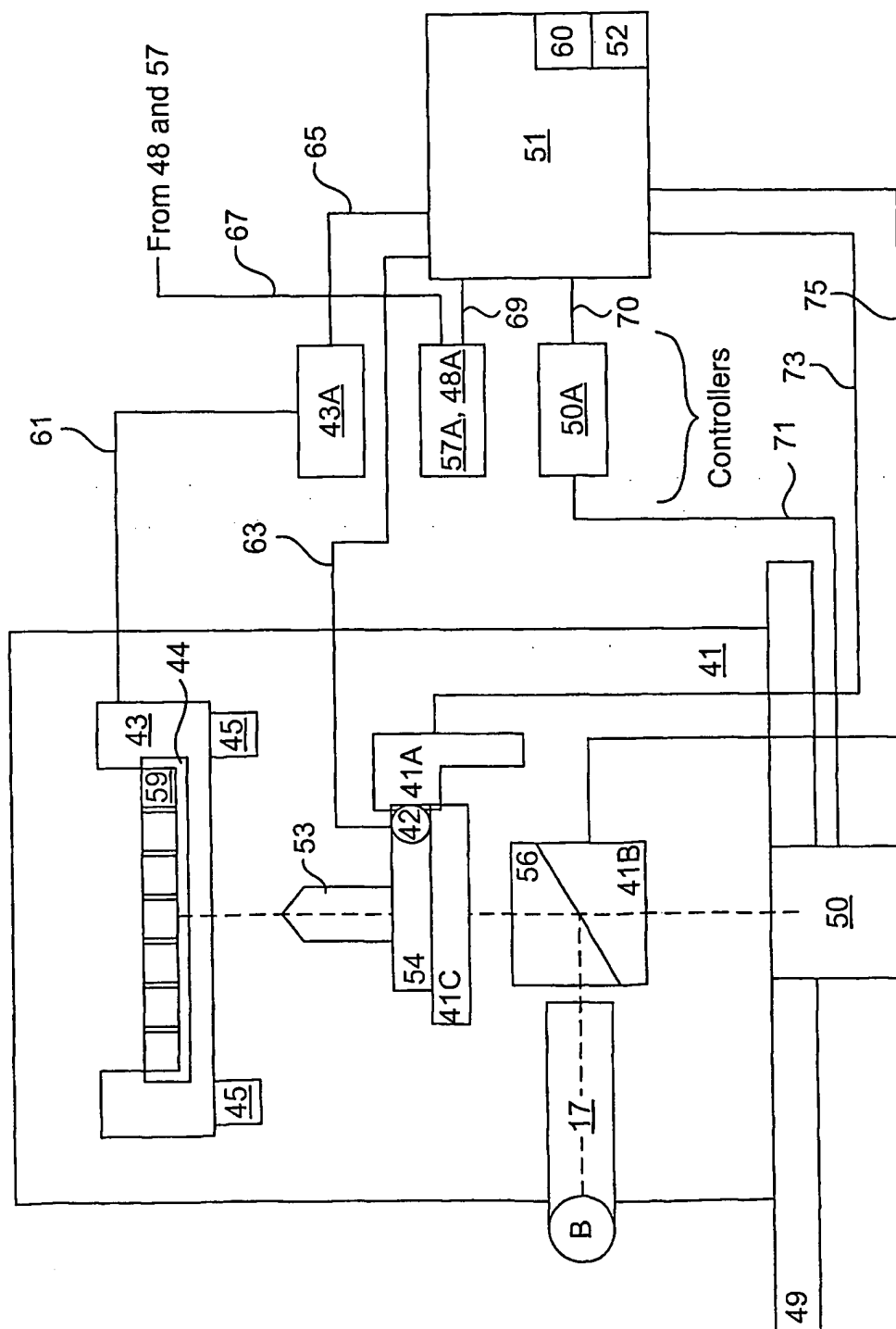


FIG. 5A

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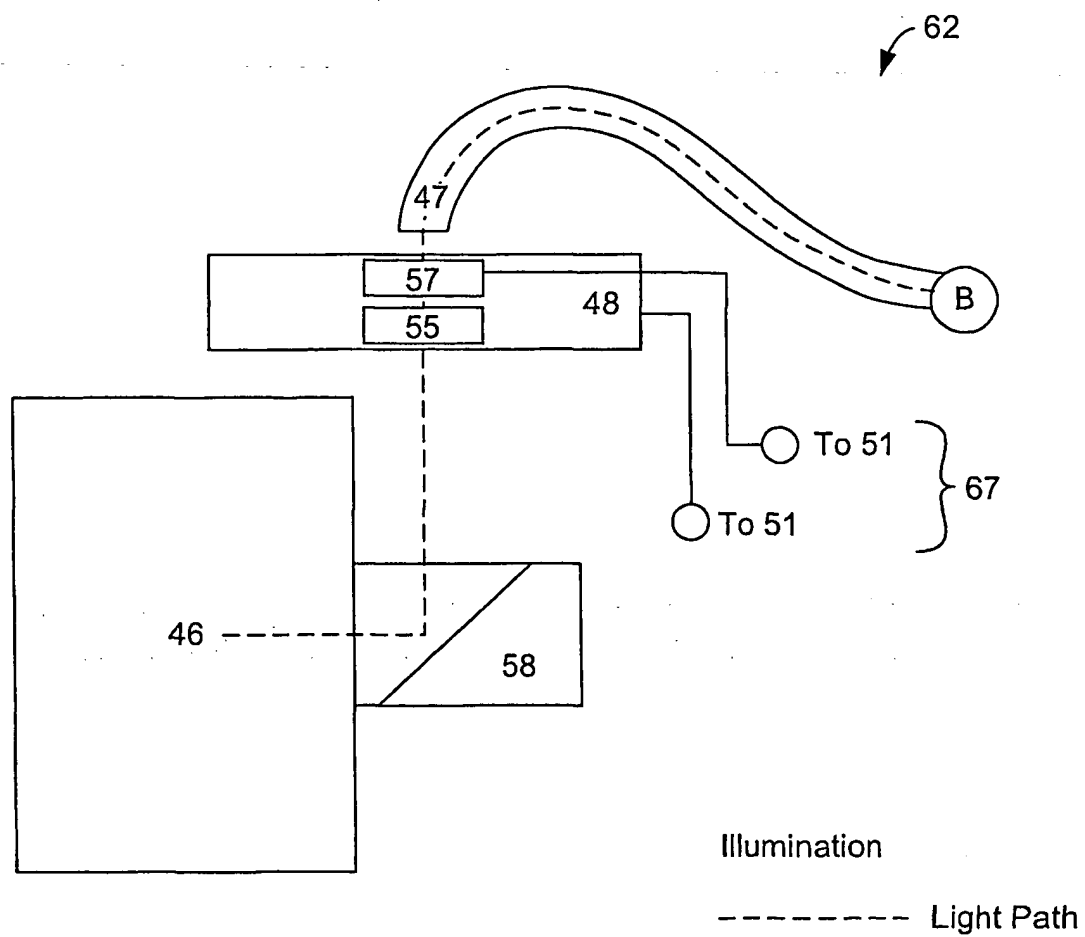


FIG. 5B

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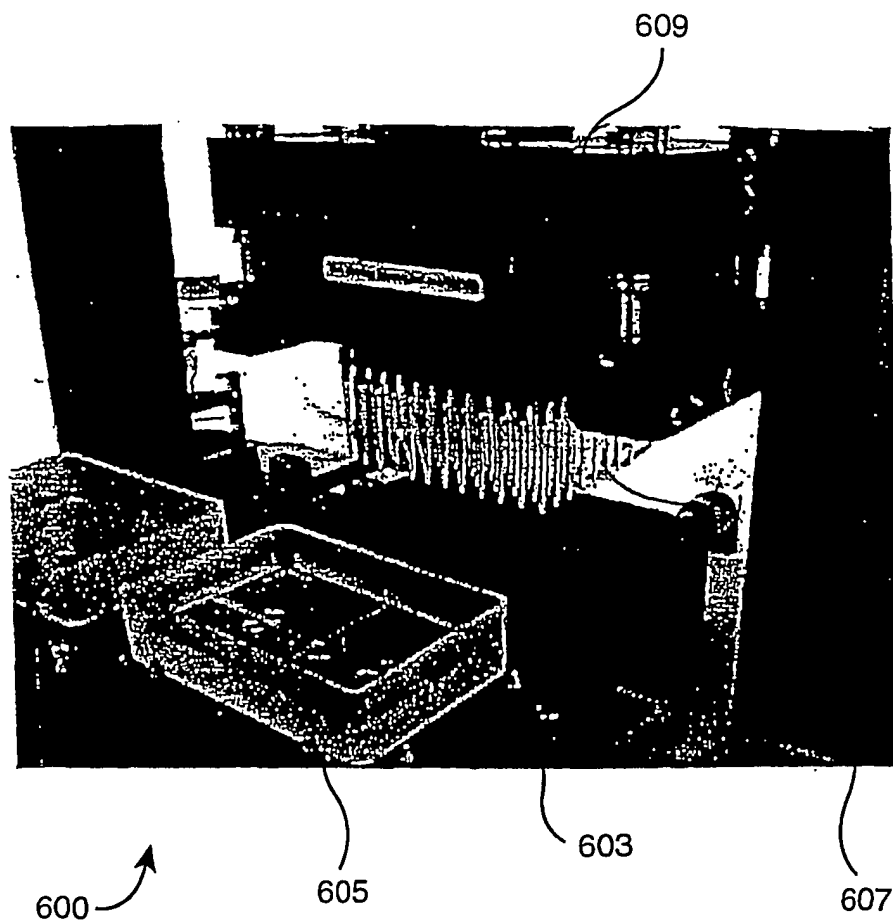


FIG. 6

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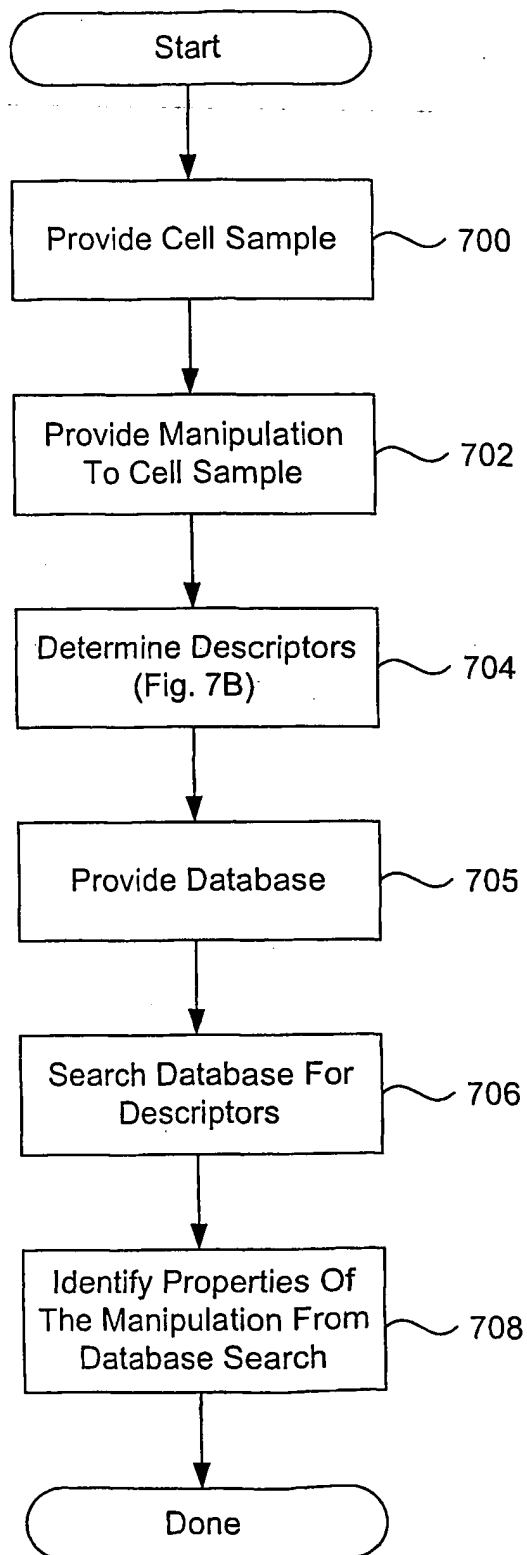


FIG. 7A

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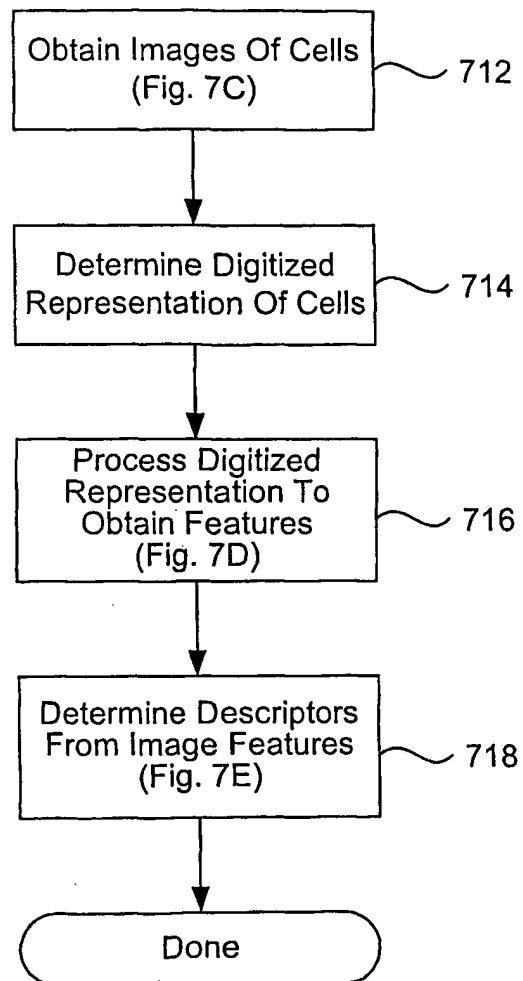


FIG. 7B
Step 704 of Fig. 7A

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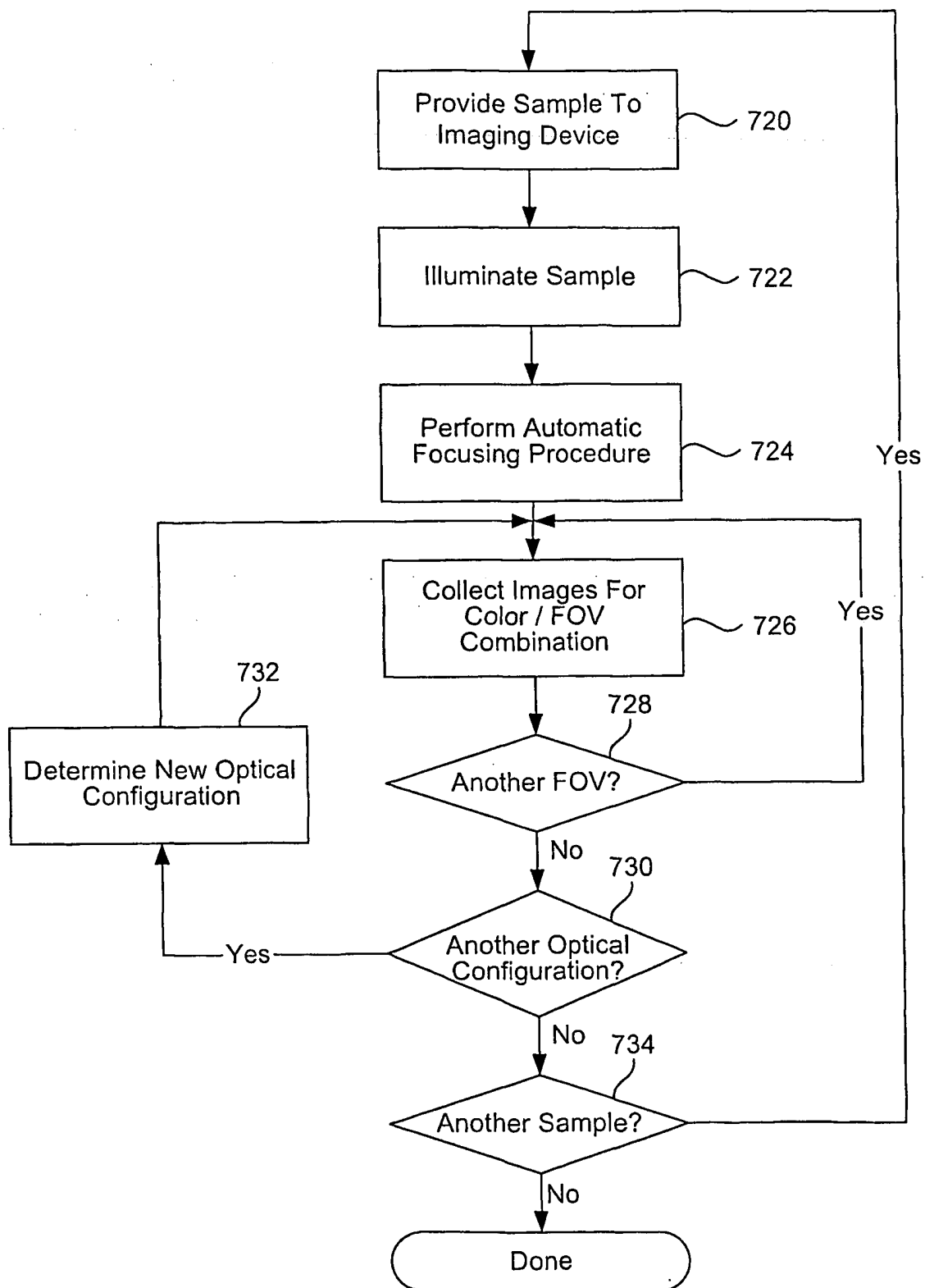


FIG. 7C

Step 714 of Fig. 7B

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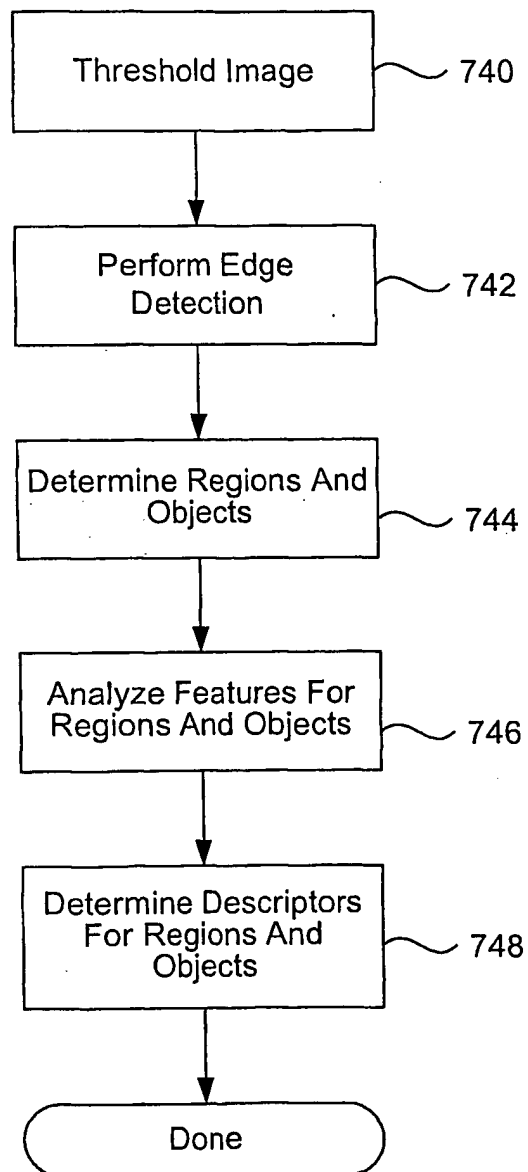


FIG. 7D
Step 716 of Fig. 7B

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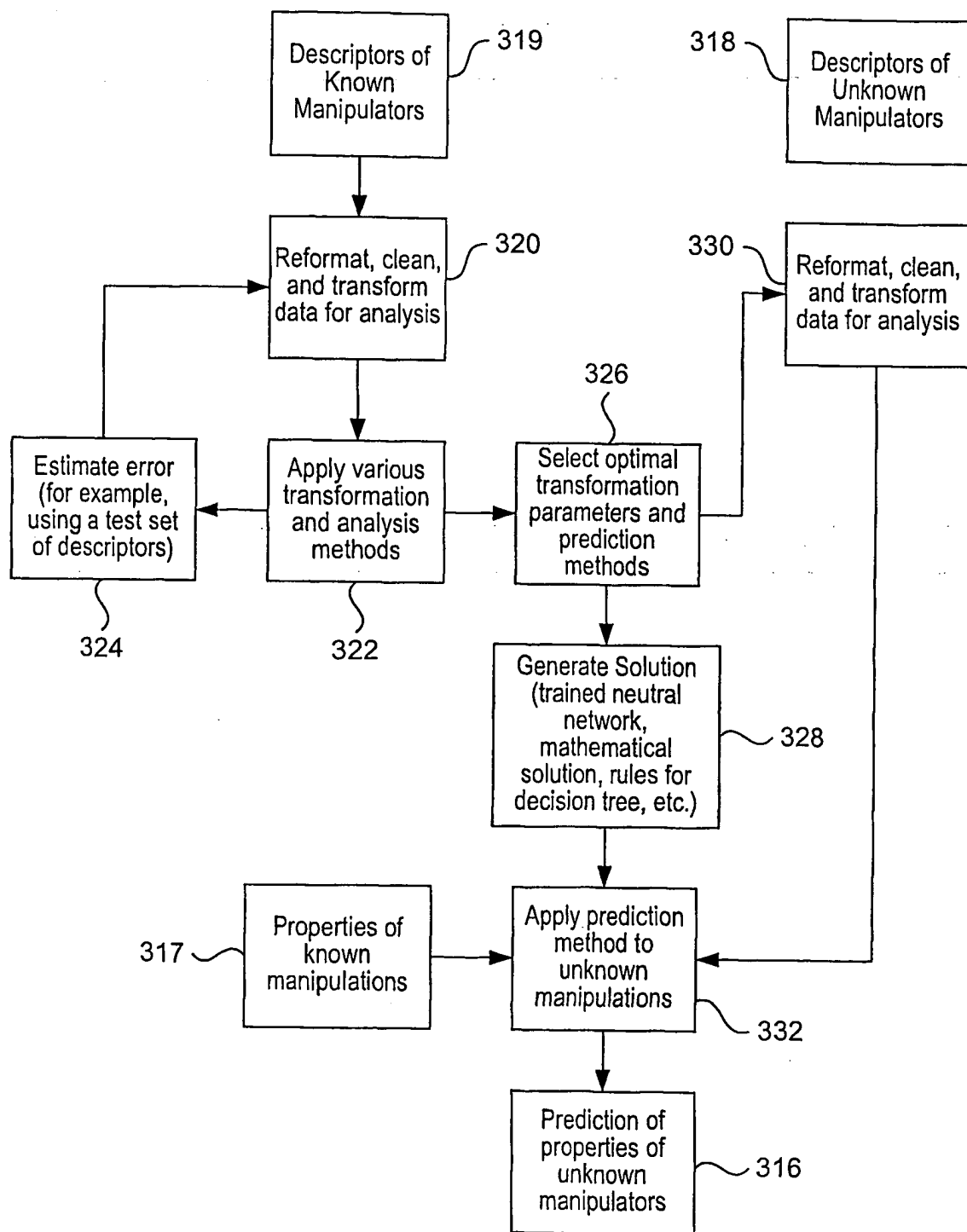


FIG. 7E

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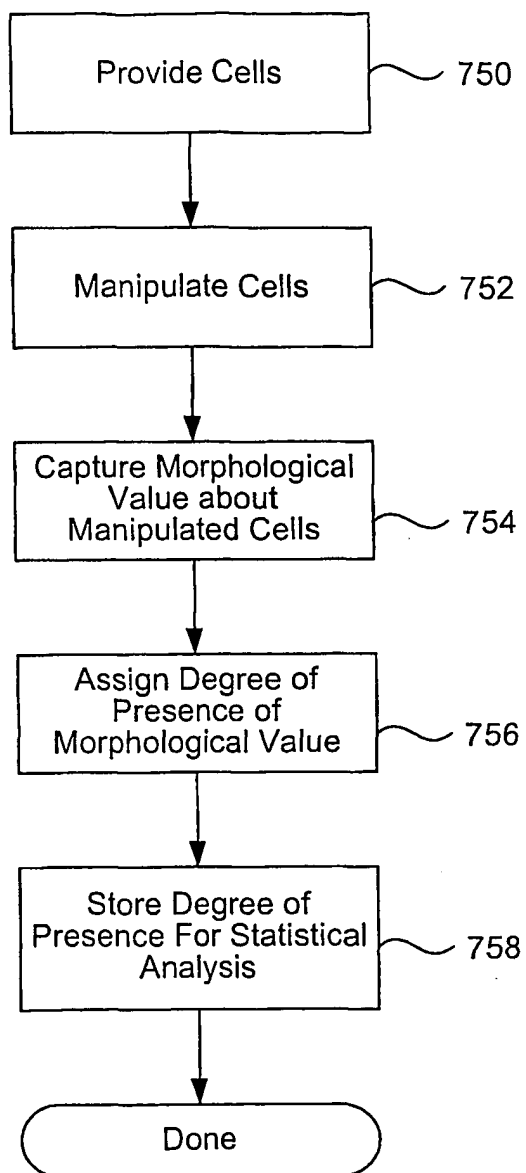


FIG. 7F

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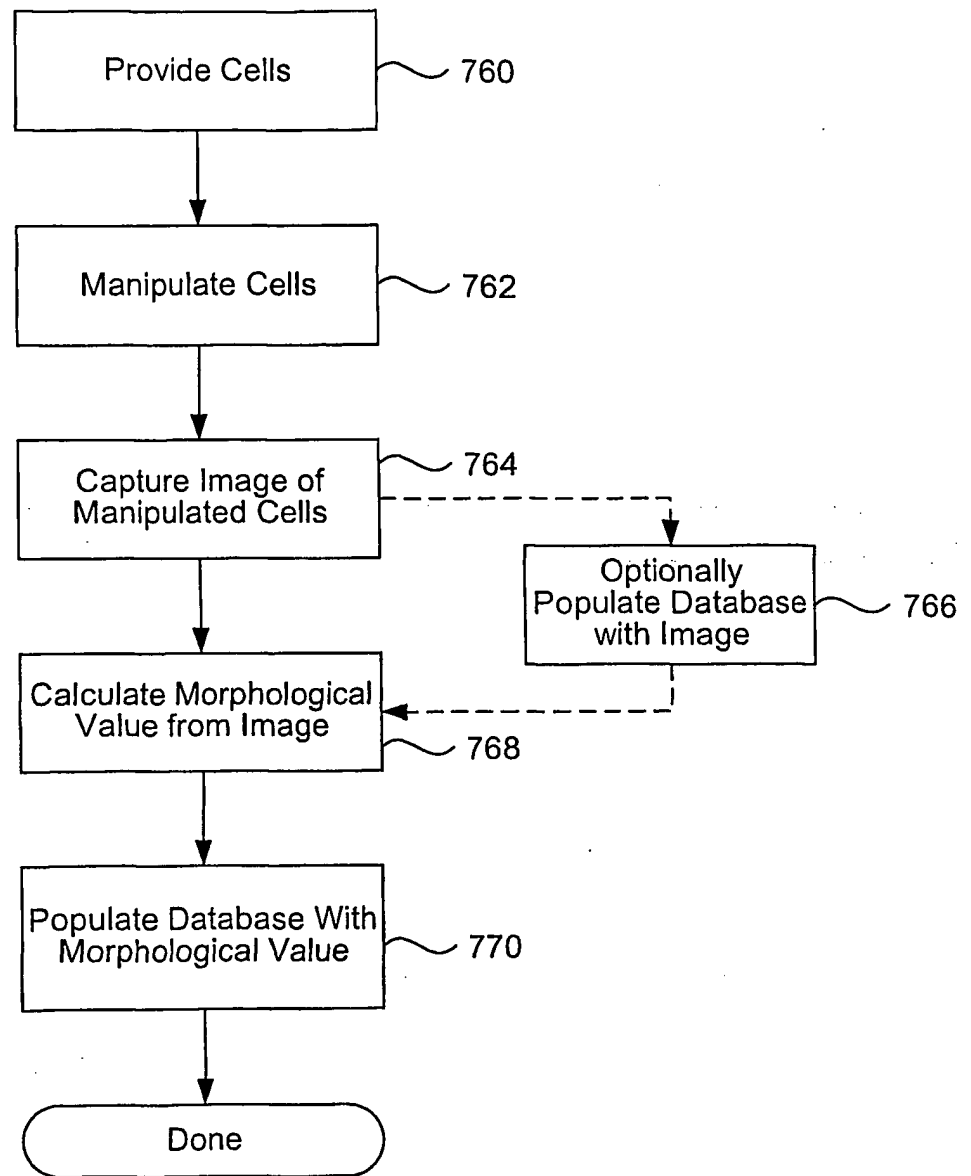


FIG. 7G

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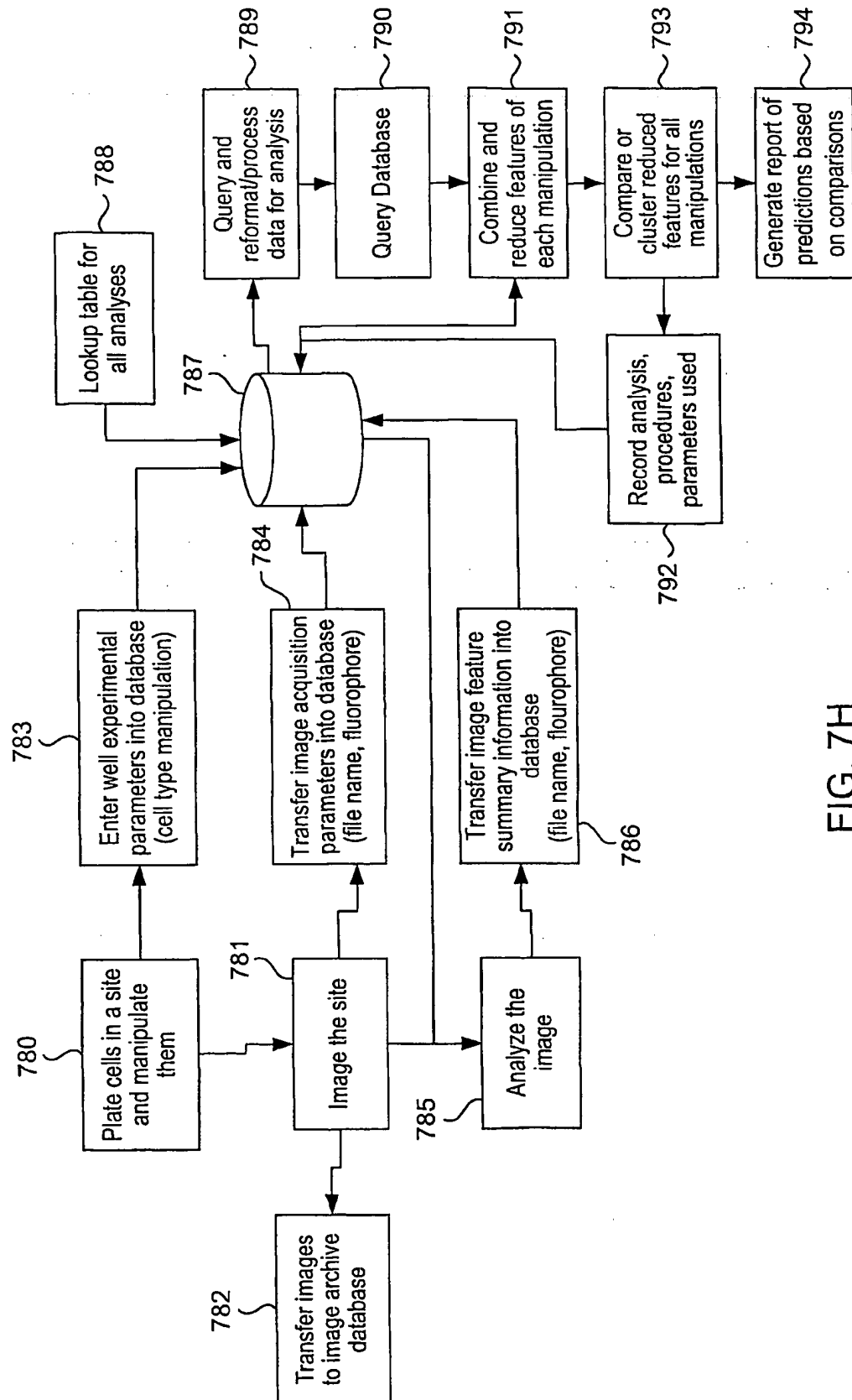


FIG. 7H

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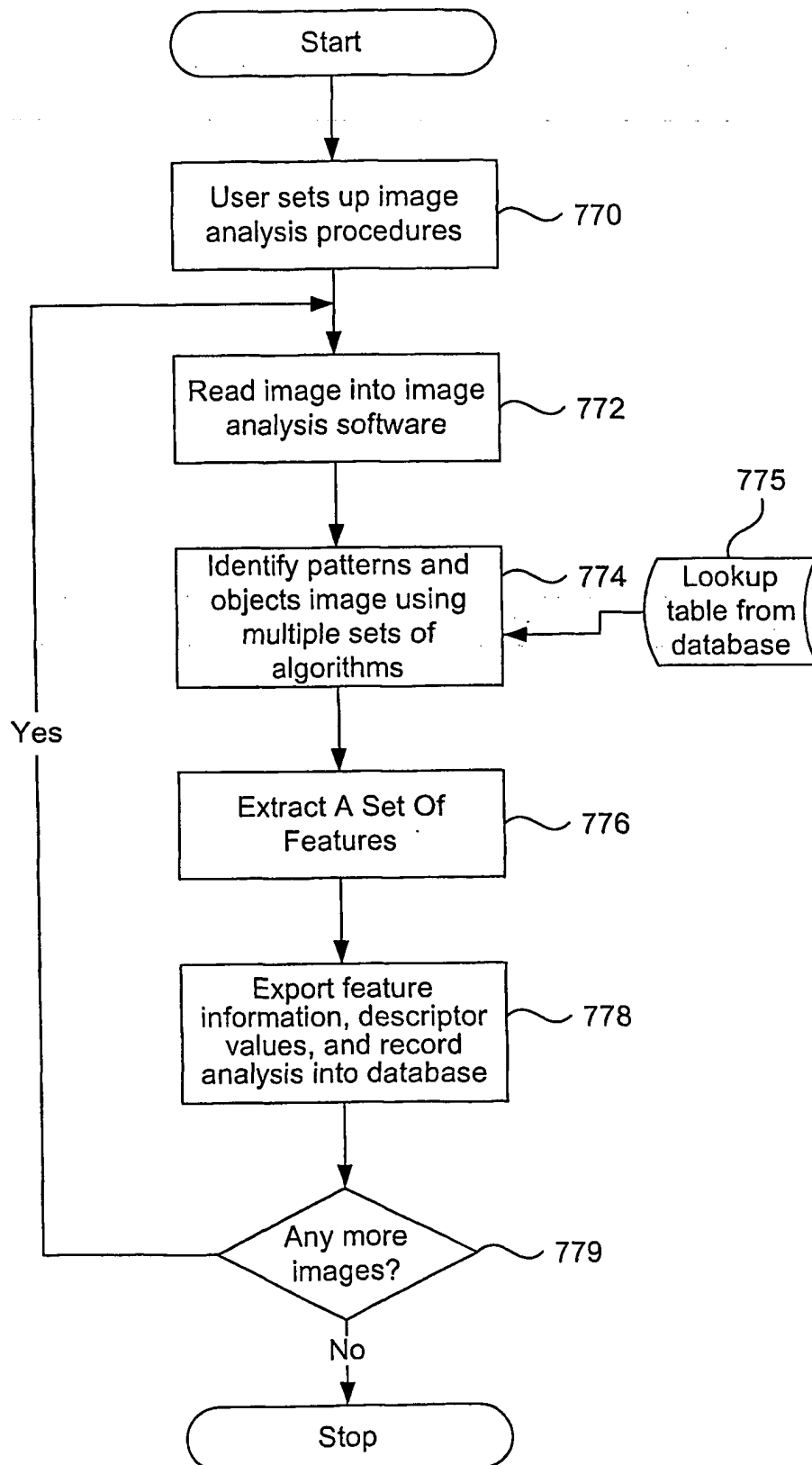


FIG. 71

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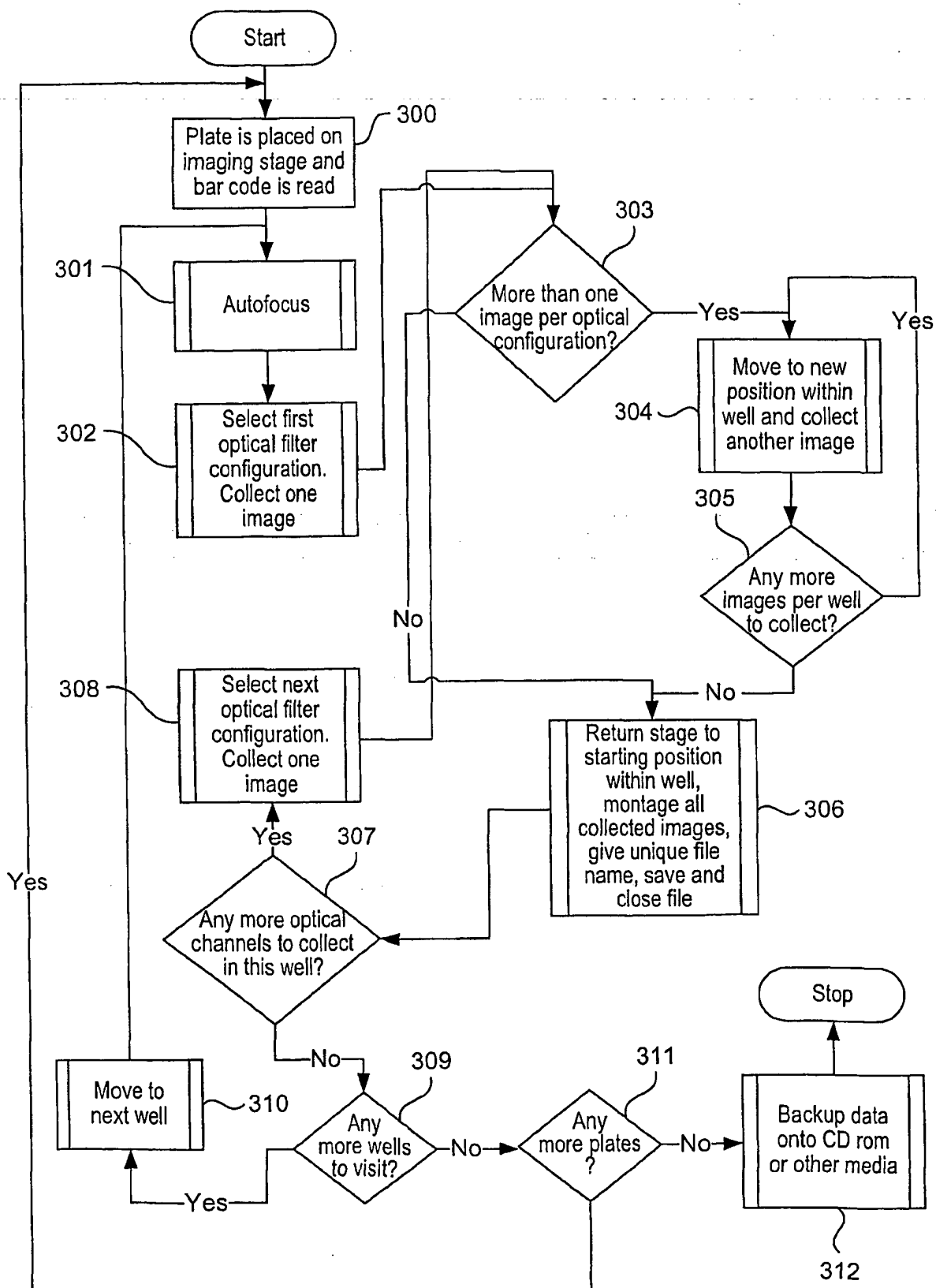


FIG. 7J

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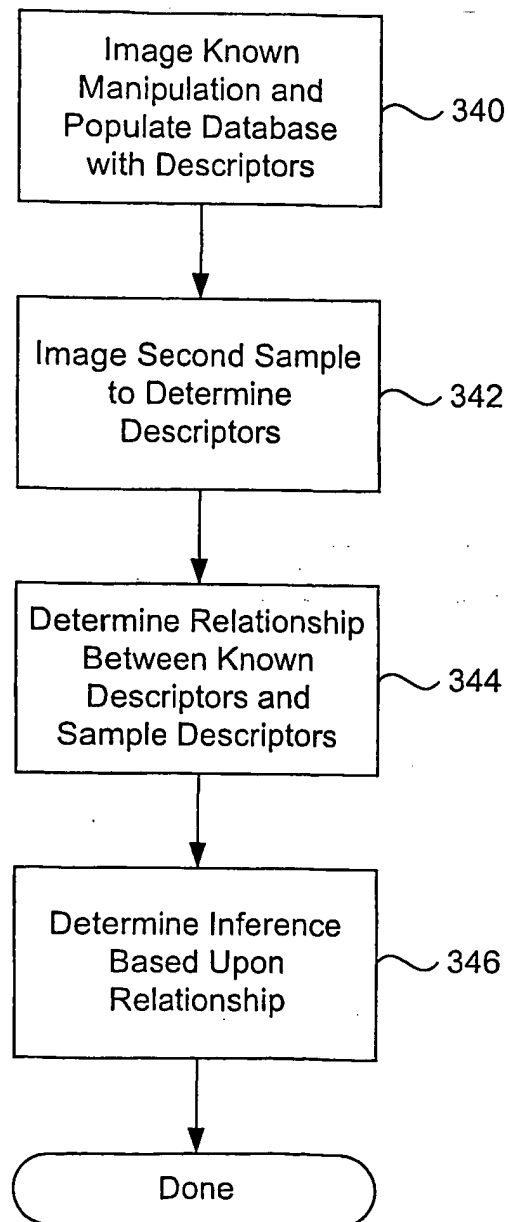


FIG. 7K

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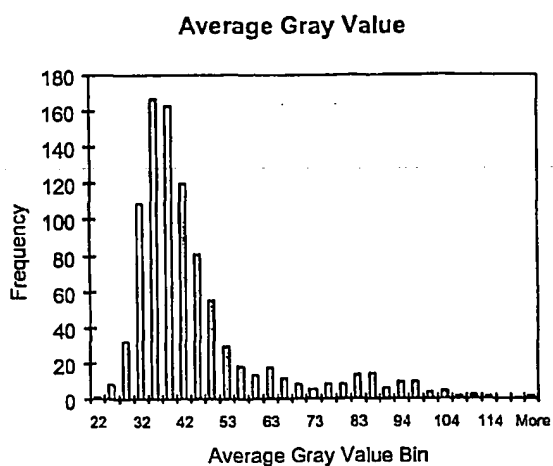


FIG. 8A

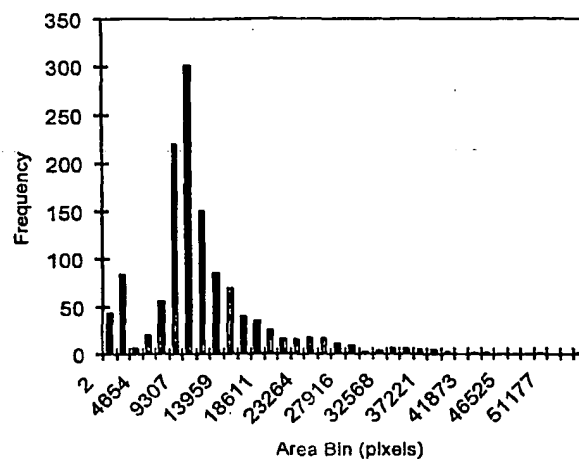


FIG. 8B

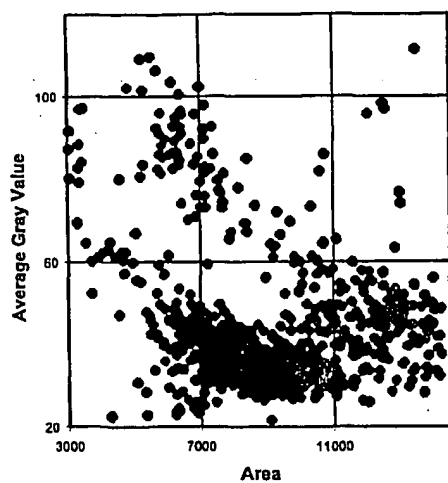


FIG. 8C

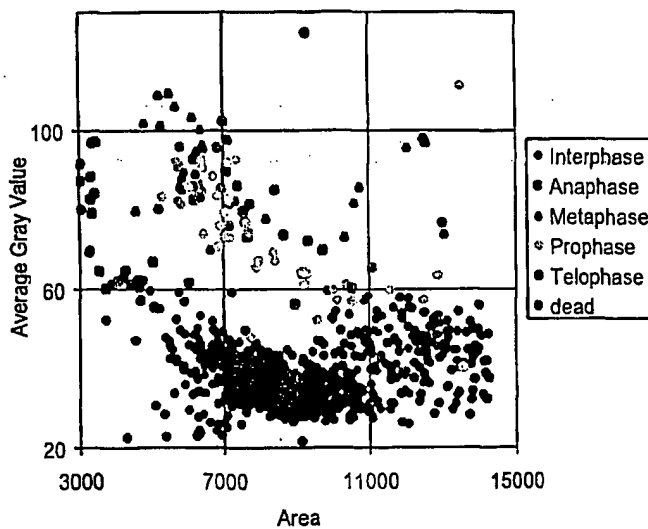


FIG. 8D

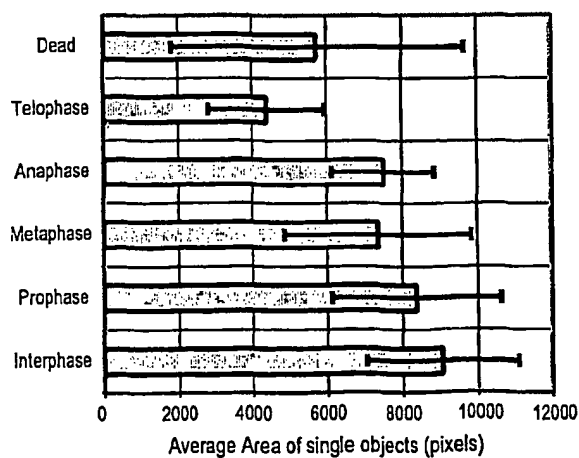


FIG. 8E

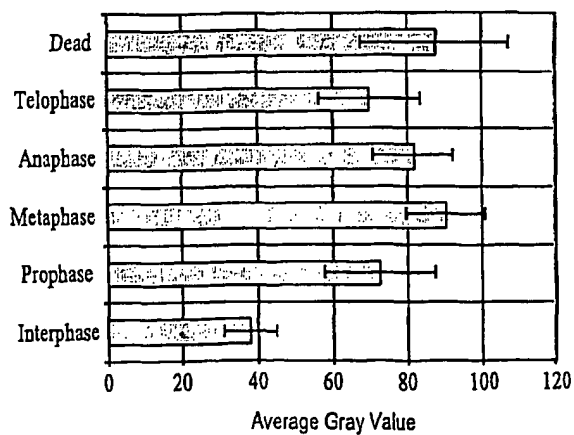


FIG. 8F

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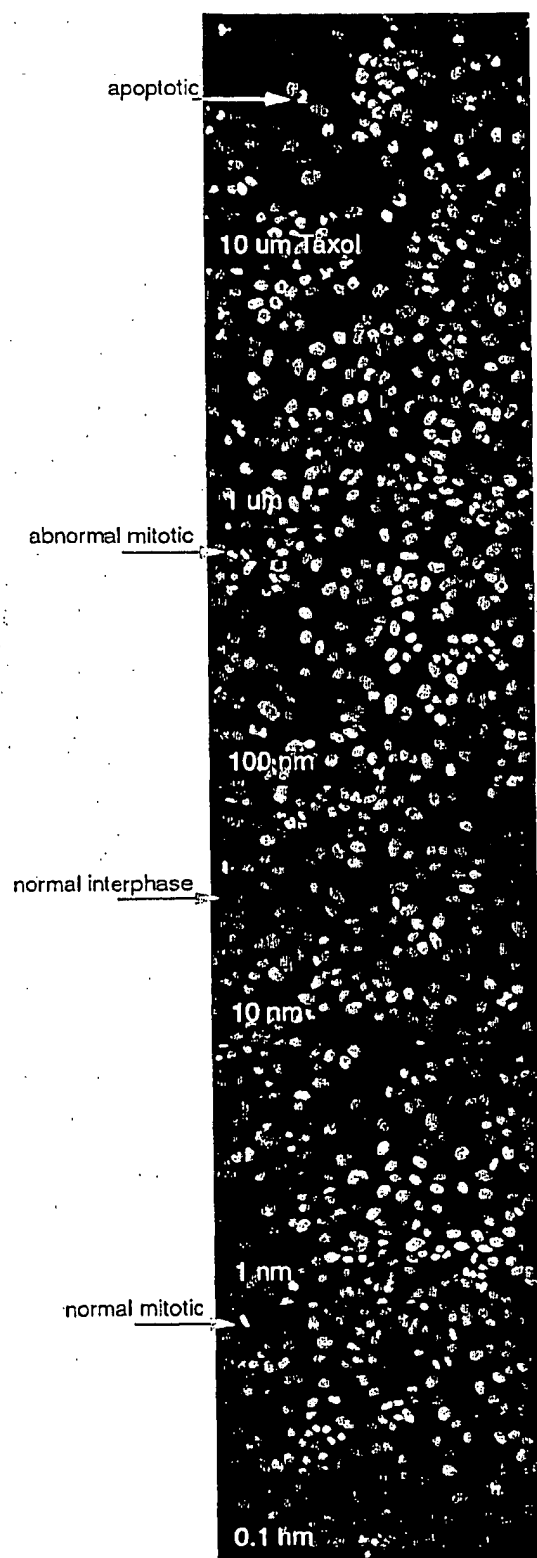


FIG. 9

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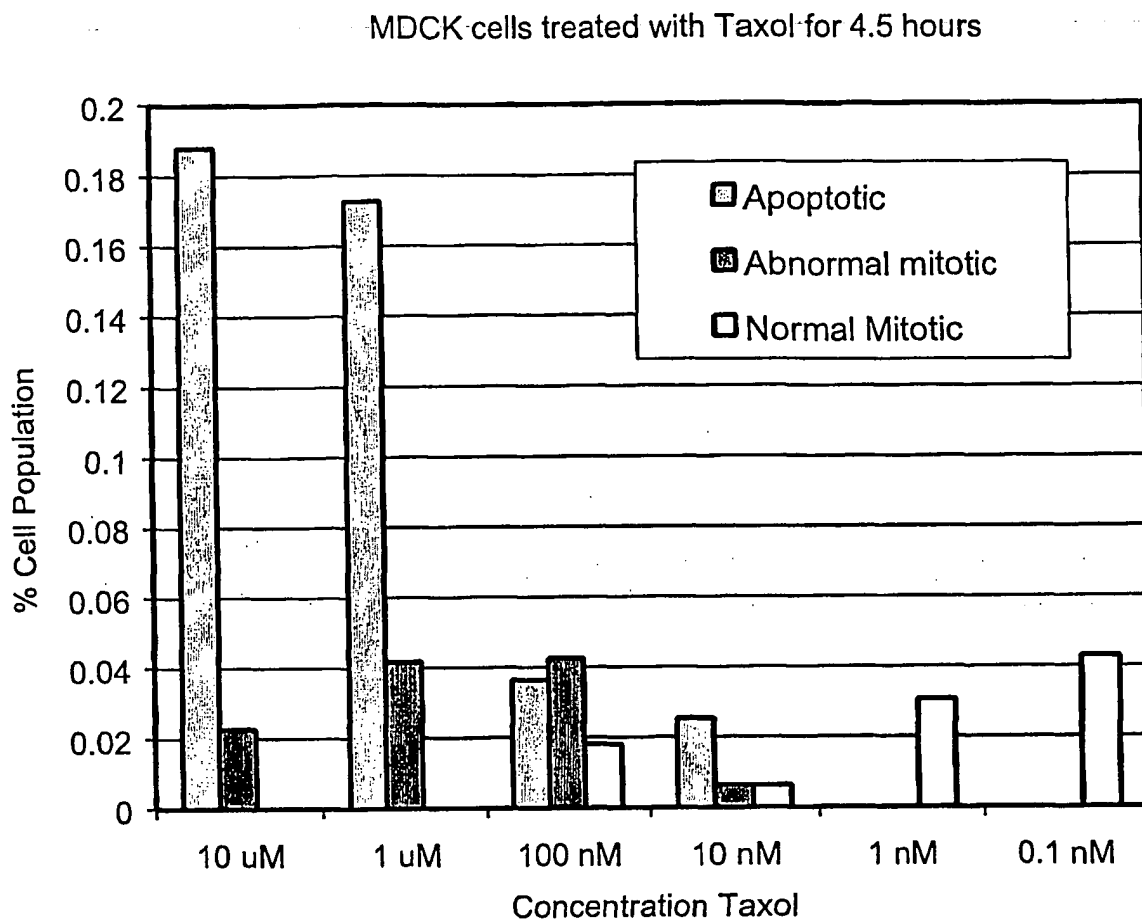


FIG. 10

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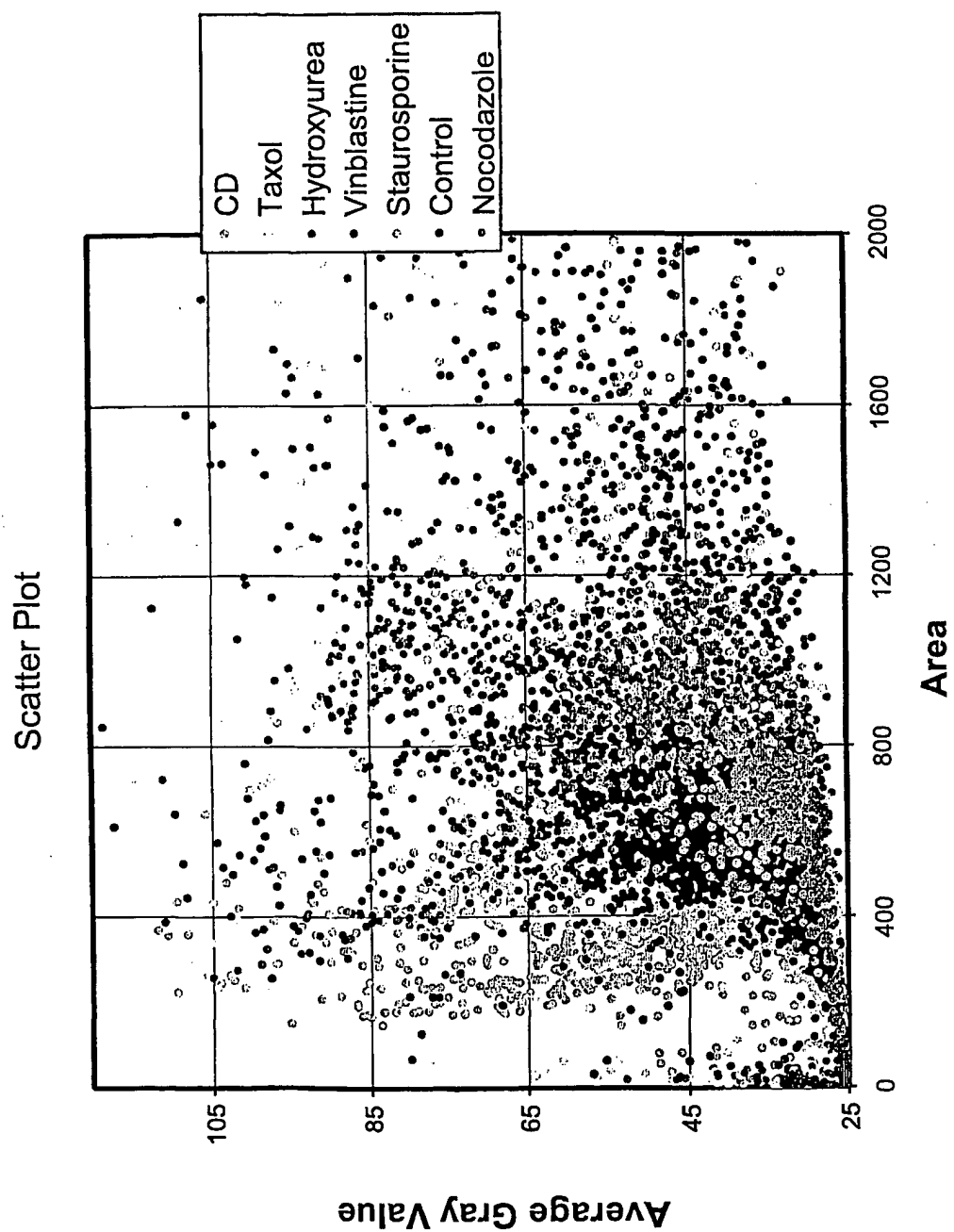


FIG. 11

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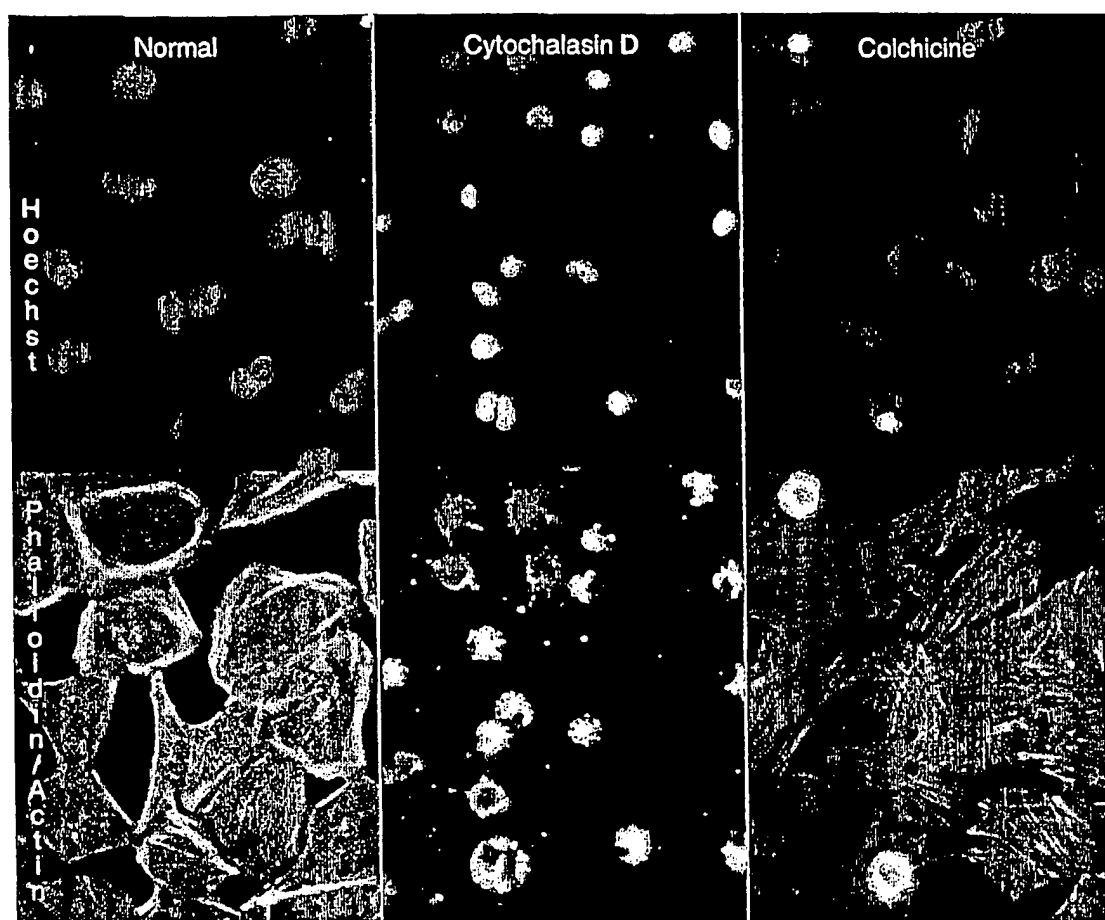


FIG. 12

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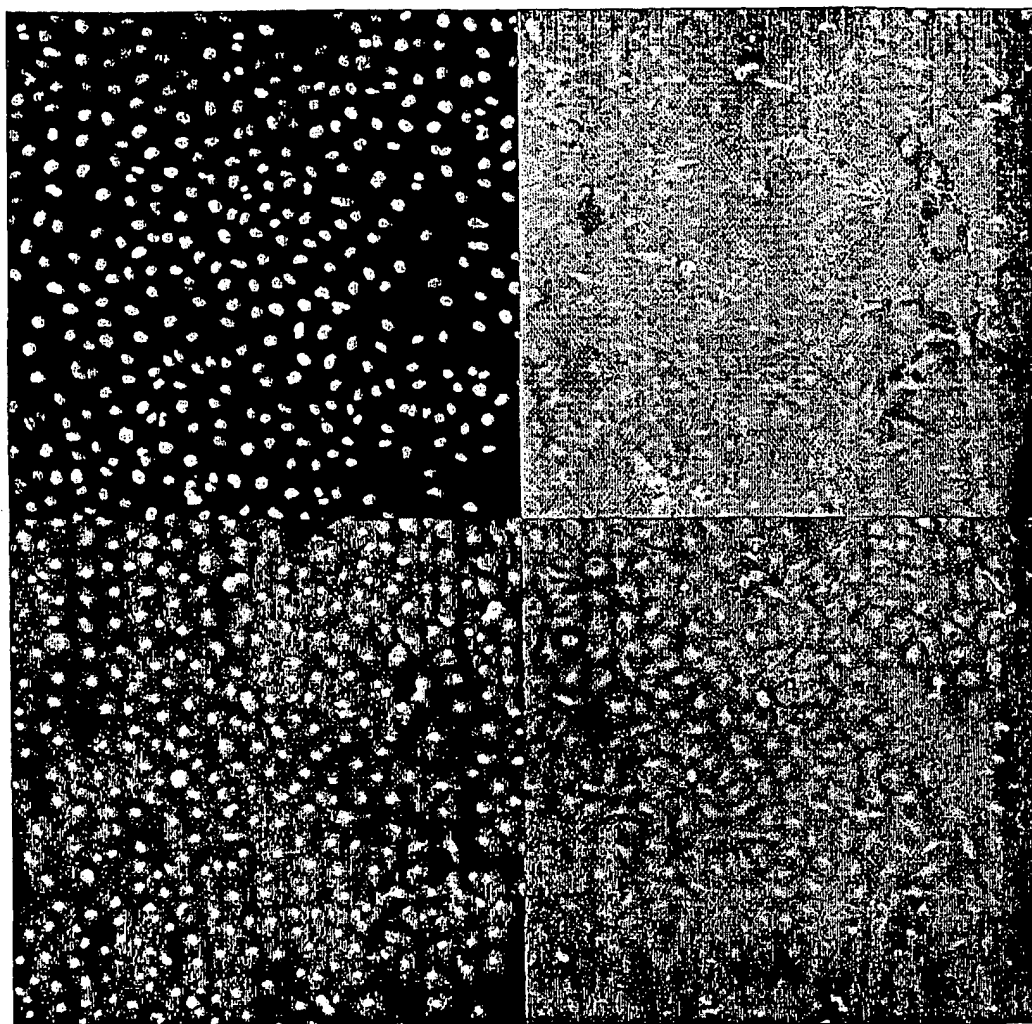


FIG. 13

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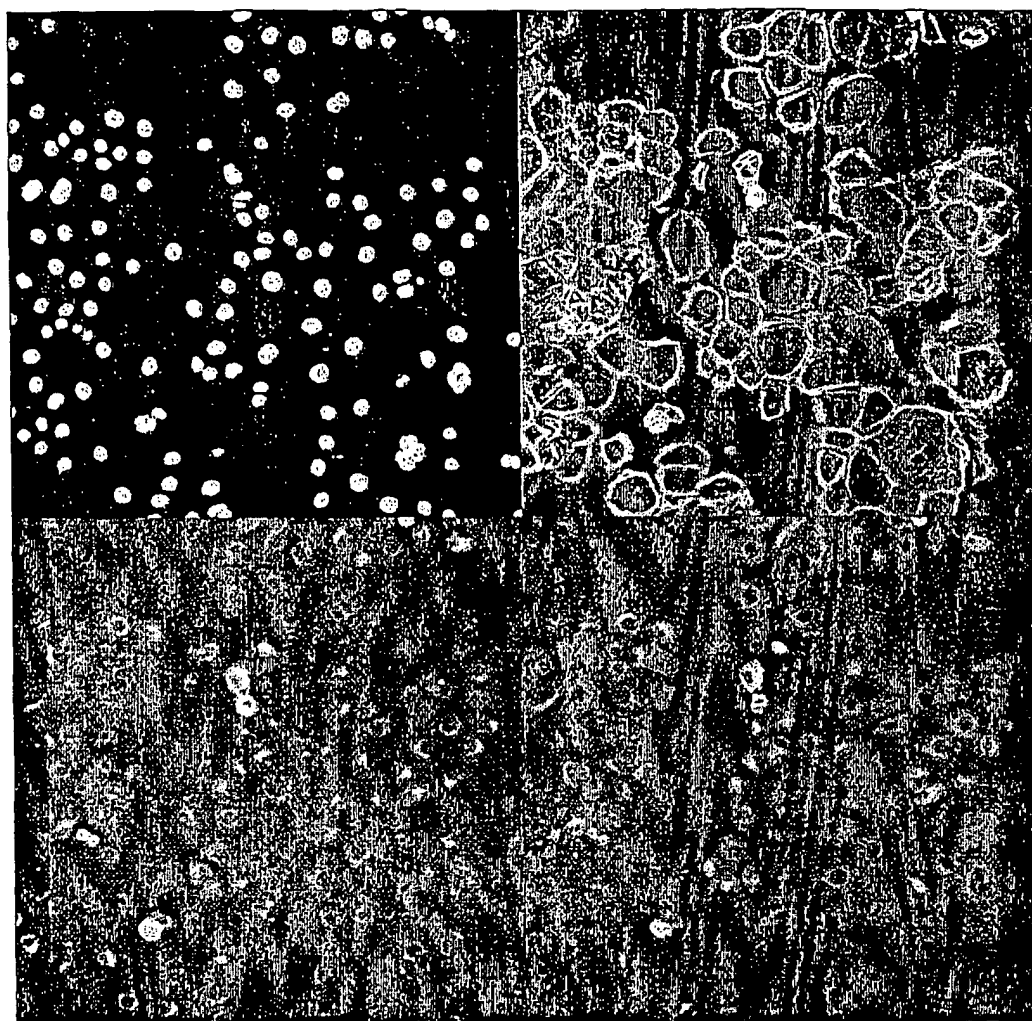


FIG. 14

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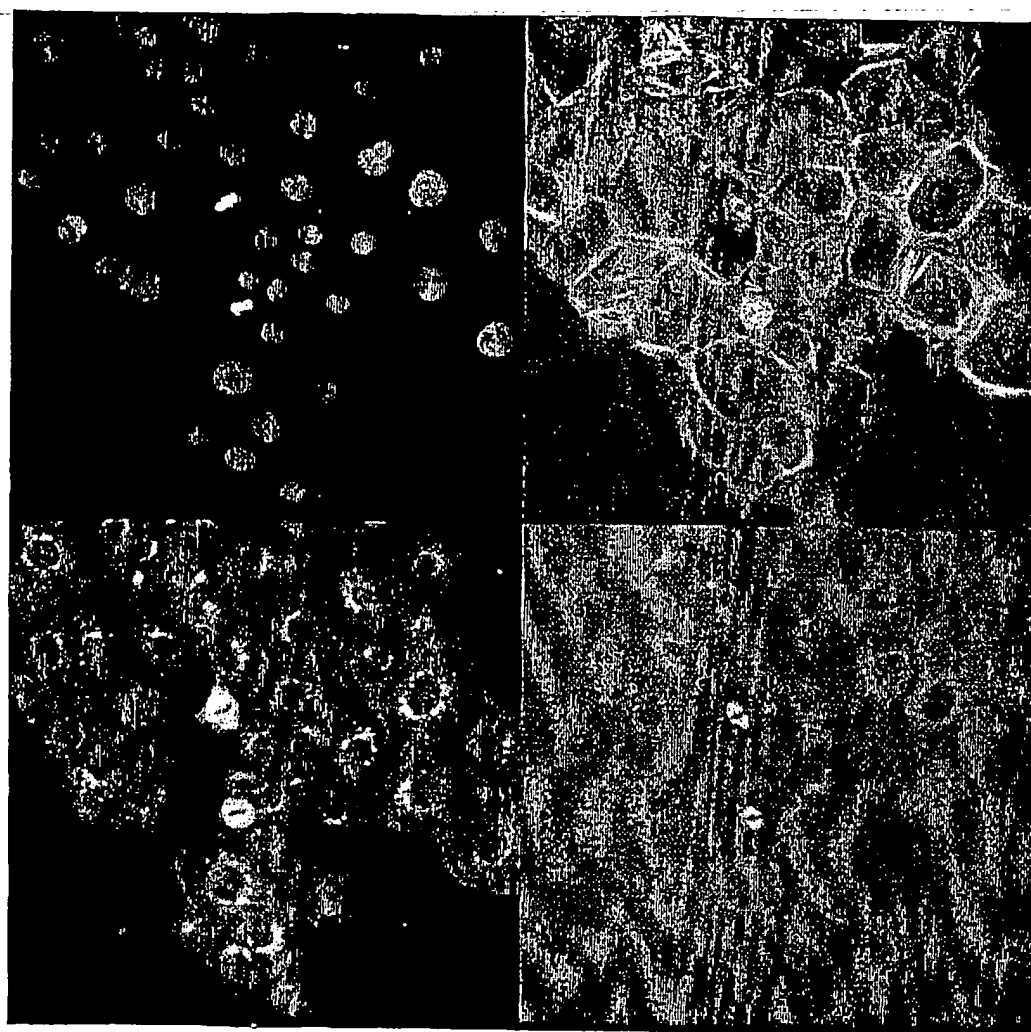


FIG. 15

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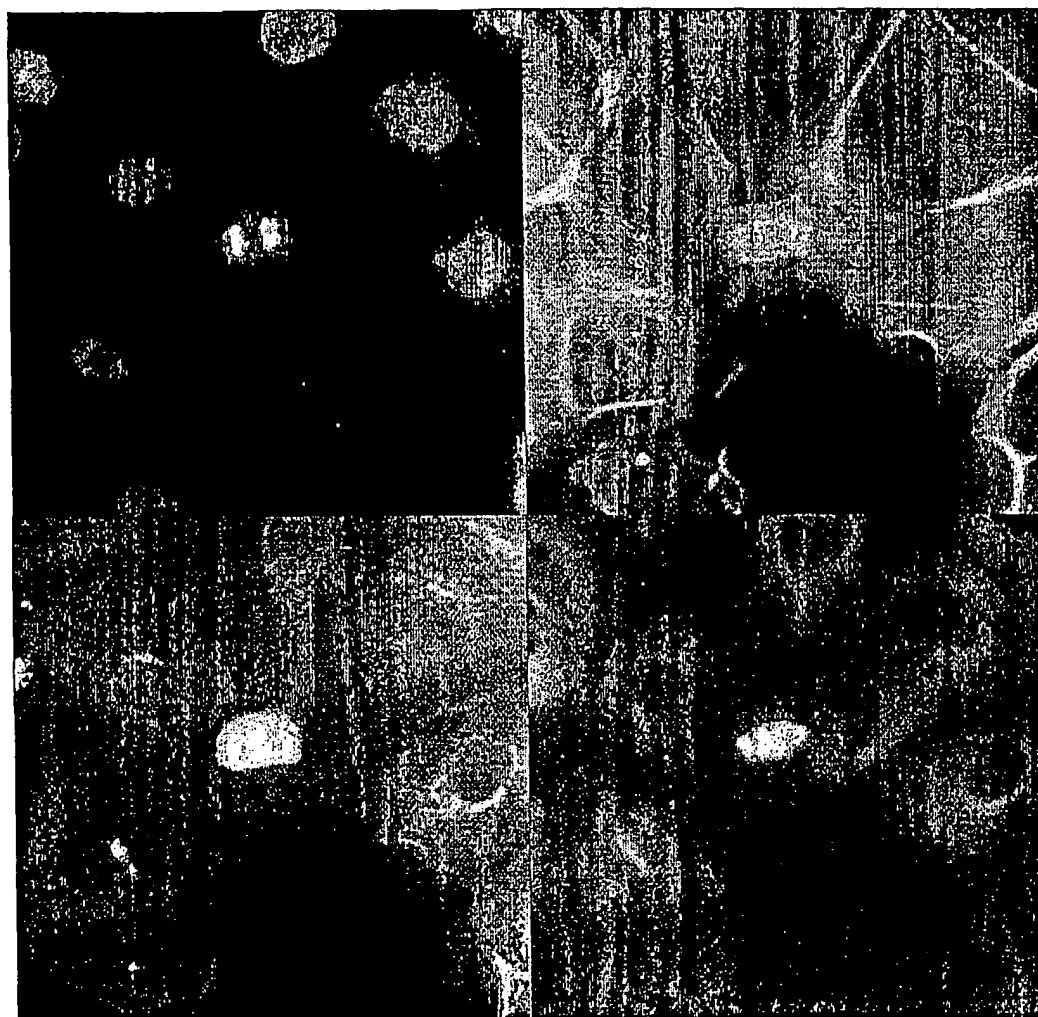


FIG. 16

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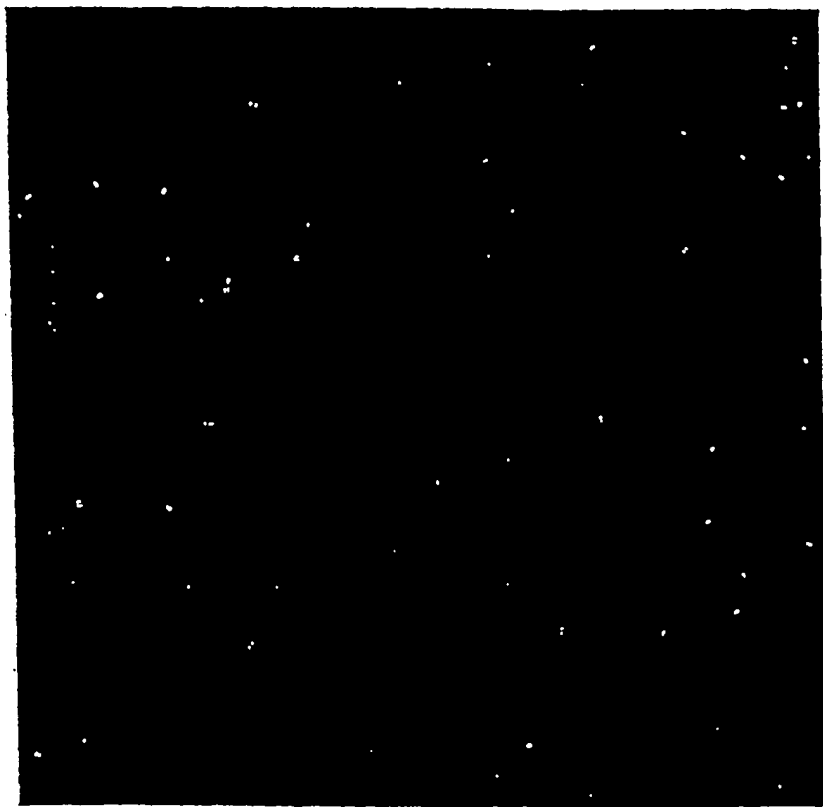


FIG. 17

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Conversion of morphometric parameters into nucleic acid code
and clustering of the resulting sequences using Neighbor
Joining method.

Compound:	Measurements																			
	Count	Area	Perimeter	Length	Breadth	Fiber length	Fiber breadth	Shape factor	Ell. form factor	Inner radius	Outer radius	Mean radius	Equiv. radius	Equiv. sphere vol.	Equiv. prolate vol.	Equiv. oblate vol.	Equiv. sphere surface area	Average gray value	Total gray value	Optical density
Control	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
Taxol	a	t	t	t	t	t	t	t	a	t	t	t	t	t	t	t	t	t	t	t
CD	c	a	a	a	t	a	t	t	c	a	a	a	a	a	a	a	a	t	a	a
Nocodazol	c	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
Staurosporine	g	g	c	a	a	t	a	a	t	g	a	a	a	t	g	g	g	a	a	t
Vinblastine	c	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	g	t	t
Hydroxyurea	g	t	t	t	t	t	t	g	t	t	t	t	t	t	t	t	t	t	c	t

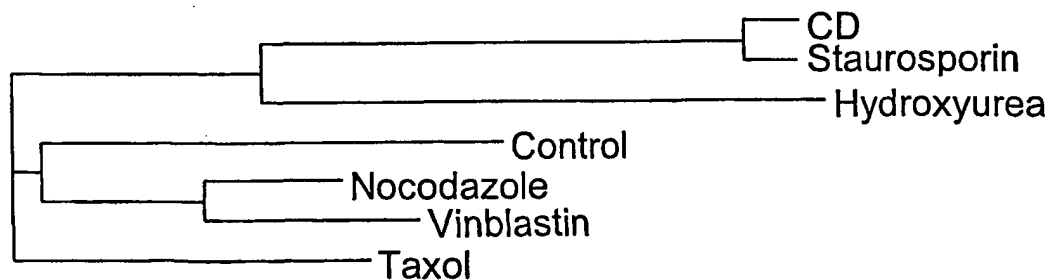


FIG. 18

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Conversion of morphometric parameters into amino acid codes and clustering of the resulting sequences using Neighbor Joining method.

	Count	Area	Perimeter	Length	Breadth	Fiber length	Fiber breadth	Shape factor	Ell. form factor	Inner radius	Outer radius	Mean radius	Equiv. radius	Equiv. sphere vol.	Equiv. prolate vol.	Equiv. oblate vol.	Equiv. sphere surface area	Average gray value	Total gray value	Optical density	Radial dispersion	Texture Difference Moment	EFA Harmonic 2, Semi-Major Axis	EFA Harmonic 2, Semi-Minor Axis	EFA Harmonic 2, Semi-Major A
Control	H	P	T	T	N	S	D	W	E	S	T	T	T	F	C	C	P	P	M	C	T	G	T	T	Y
Taxol	G	F	M	M	P	M	P	H	G	S	M	M	W	C	F	P	F	R	C	M	M	H	M	P	S
CD	F	G	G	G	M	G	M	K	A	G	G	G	G	G	G	G	G	H	G	G	G	M	G	V	H
Nocodazol	W	F	M	M	W	M	P	T	R	S	M	M	M	F	M	W	F	M	M	R	M	M	M	F	G
Staurosporine	N	V	A	G	G	M	G	G	Y	V	G	G	G	M	V	V	V	G	G	H	G	M	G	G	V
Vinblastine	F	W	W	M	W	W	C	W	D	S	M	W	W	M	M	M	W	M	V	E	M	M	M	F	P
Hydroxyurea	S	H	H	H	H	H	H	V	H	H	H	H	H	H	H	H	H	H	H	A	H	G	H	H	D

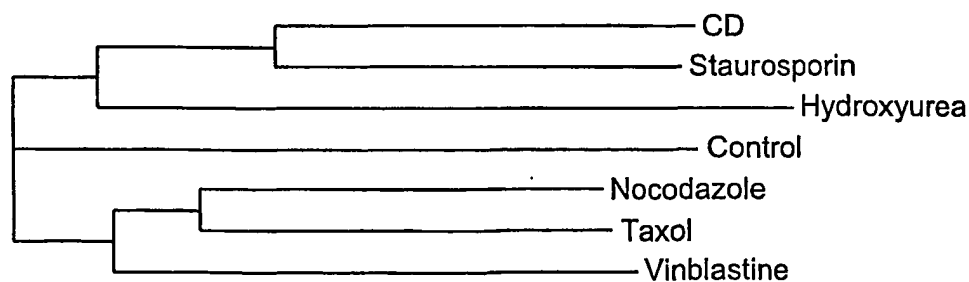


FIG. 19

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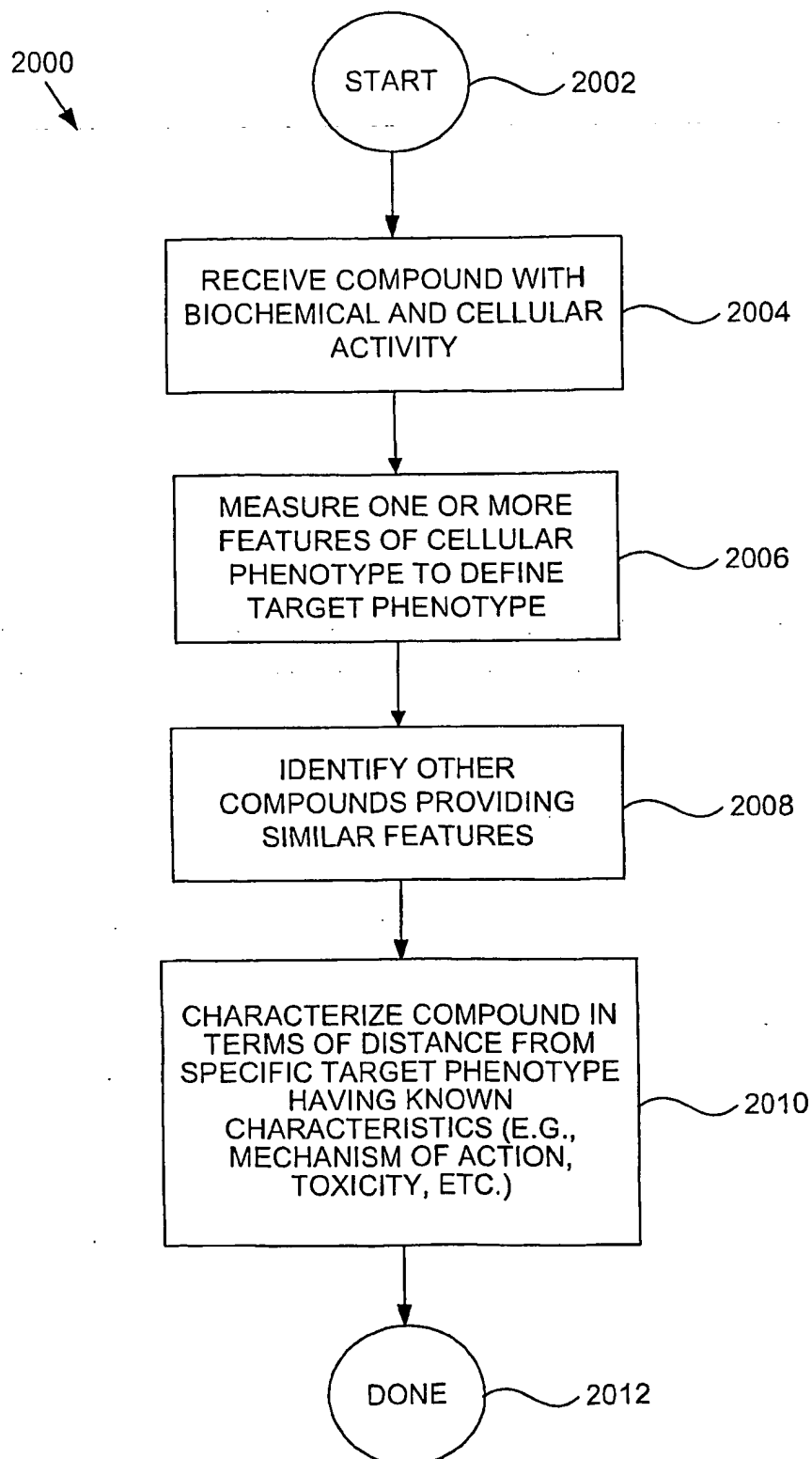


FIG. 20

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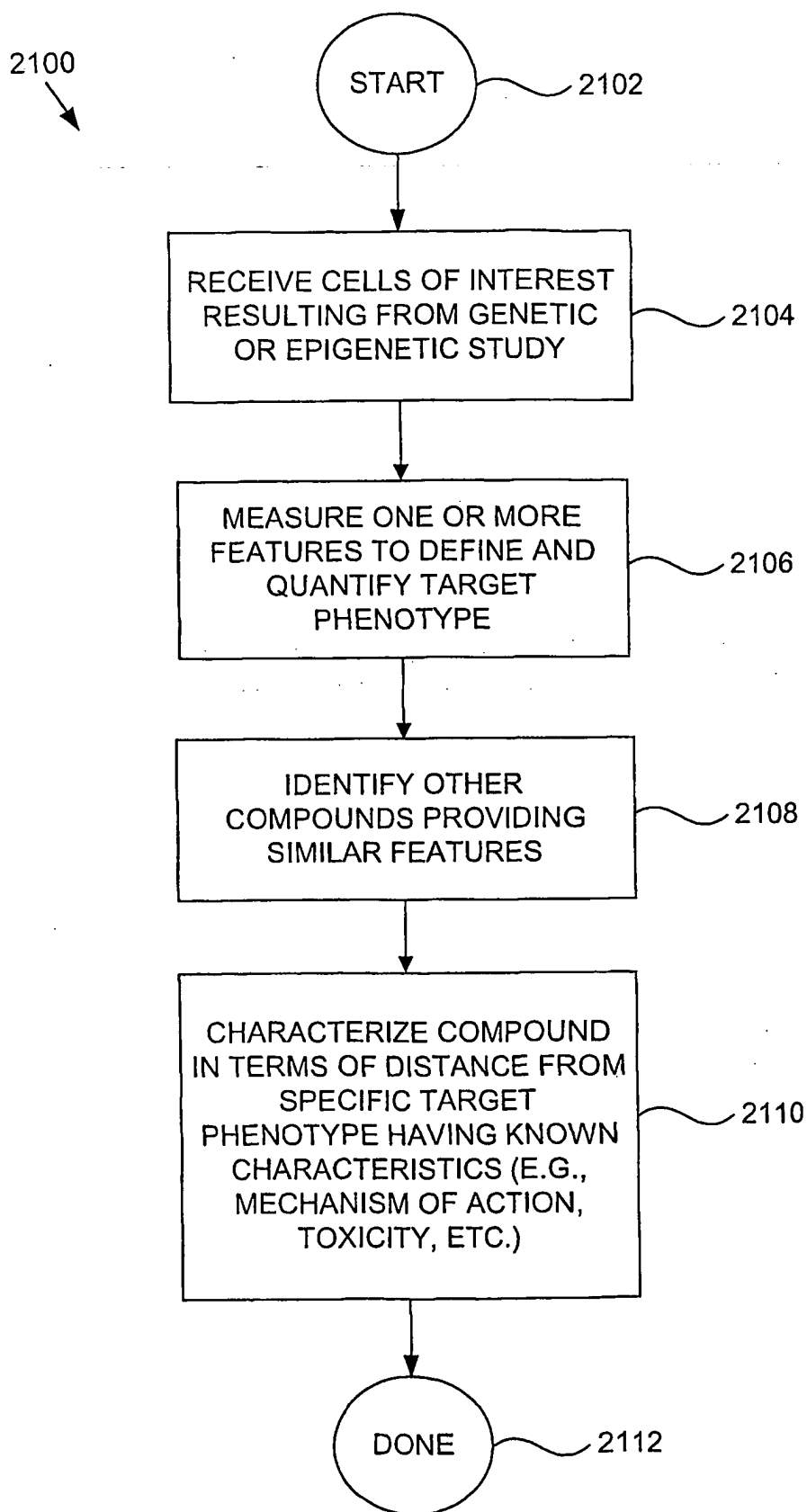


FIG. 21

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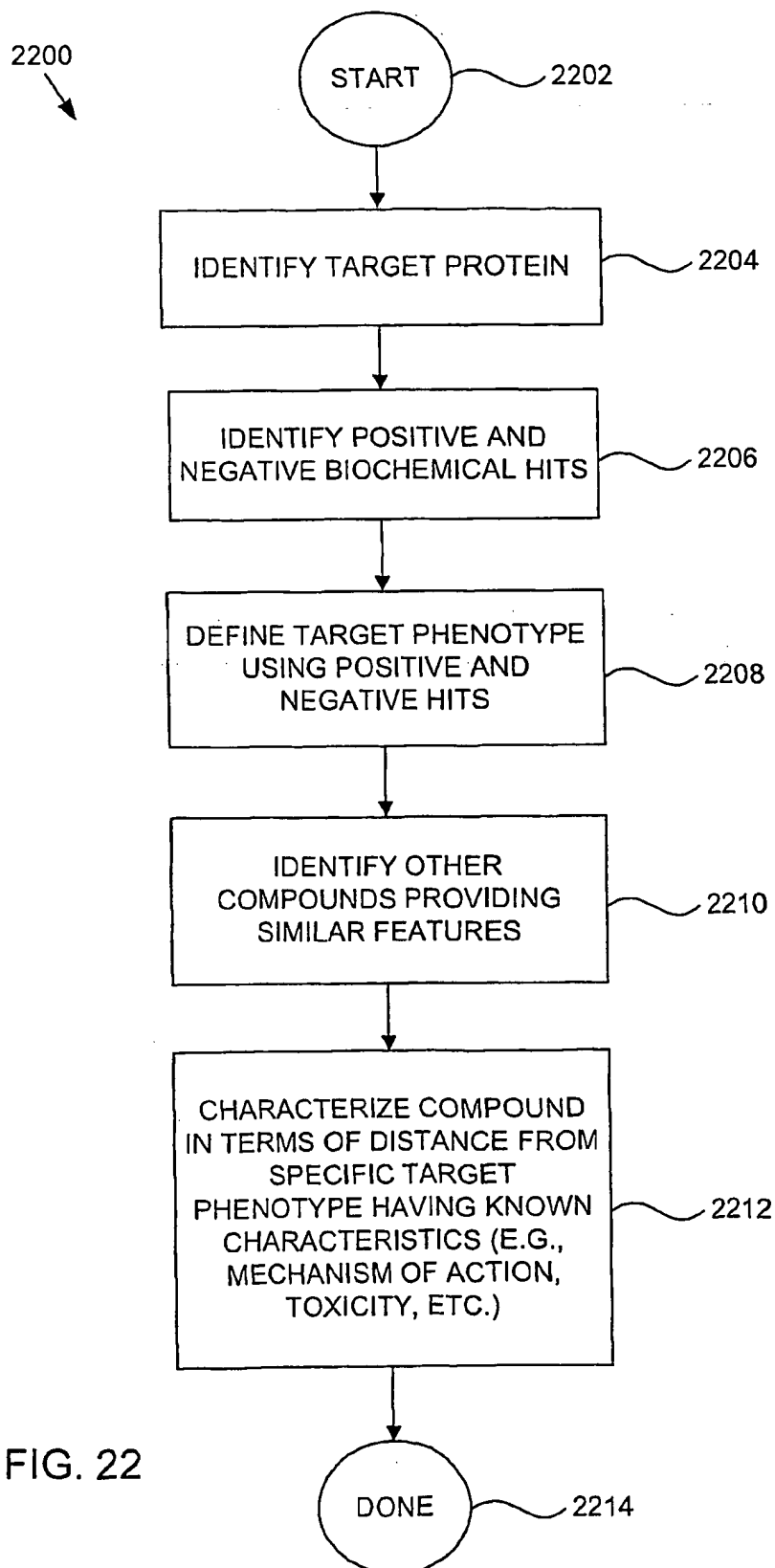


FIG. 22

INTERNATIONAL SEARCH REPORT

Intel. .onal Application No

PCT/US 00/13154

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G06F19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G06F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 38490 A (BIODX INC ;DUNLAY R TERRY (US); GOUGH ALBERT H (US); GIULIANO KENN) 3 September 1998 (1998-09-03) cited in the application	1-6, 24-27
Y	page 1; claims 1-43	7-23
X	WO 98 45704 A (TULLIN SOEREN ;KASPER ALMHOLT (DK); NOVONORDISK AS (DK); SCUDDER K) 15 October 1998 (1998-10-15) abstract; claims 1-3,22,73,80,81,86	1-6, 24-27
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Z document member of the same patent family

Date of the actual completion of the international search

17 November 2000

Date of mailing of the international search report

24/11/2000

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Fax (+31-70) 340-3336

Authorized officer

Filloy García, E

INTERNATIONAL SEARCH REPORT

Inter. .onal Application No

PCT/US 00/13154

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MONTIRONI R ET AL: "COMPUTED CELL CYCLE AND DNA HISTOGRAM ANALYSES IN IMAGE CYTOMETRY IN BREAST CANCER" JOURNAL OF CLINICAL PATHOLOGY, GB, LONDON, vol. 46, no. 9, 1 September 1993 (1993-09-01), pages 795-800, XP000644549 ISSN: 0021-9746 abstract ----	7-13
Y	WO 97 40055 A (DOW CHEMICAL CO ;UNIV TEXAS TECH (US)) 30 October 1997 (1997-10-30) page 18, line 26 - line 32 ----	14-23
P,X	WO 99 39184 A (HARTMANN THOMAS ;RIBOZYME PHARM INC (US)) 5 August 1999 (1999-08-05) the whole document ----	1-6, 24-27
P,X	WO 00 17643 A (CELLOMICS INC ;DUNLAY R TERRY (US); GOUGH ALBERT H (US); RUBIN RIC) 30 March 2000 (2000-03-30) the whole document ----	1-6, 24-27
E	WO 00 50872 A (CELLOMICS INC ;KAPUR RAVI (US); GIULIANO KENNETH A (US)) 31 August 2000 (2000-08-31) the whole document ----	1-6, 24-27
A	GIULIANO K A ET AL: "Fluorescent-protein biosensors: new tools for drug discovery" TRENDS IN BIOTECHNOLOGY, GB, ELSEVIER PUBLICATIONS, CAMBRIDGE, vol. 16, no. 3, 1 March 1998 (1998-03-01), pages 135-140, XP004108592 ISSN: 0167-7799 page 139, left-hand column, paragraph 4 -right-hand column, paragraph 3 -----	1-27